ESCOLA UNIVERSITÁRIA VASCO DA GAMA

MESTRADO INTEGRADO EM MEDICINA VETERINÁRIA

A SURVEY OF GASTROINTESTINAL PARASITES AND TRICHINELLA SPP. IN WILD CARNIVORES OF PORTUGAL

Ana Isabel Pereira Martins

Coimbra, Julho 2018

ESCOLA UNIVERSITÁRIA VASCO DA GAMA

MESTRADO INTEGRADO EM MEDICINA VETERINÁRIA

A SURVEY OF GASTROINTESTINAL PARASITES AND TRICHINELLA SPP. IN WILD CARNIVORES OF PORTUGAL

Coimbra

Julho 2018

Ana Isabel Pereira Martins

Aluna do Mestrado Integrado em Medicina Veterinária

Constituição do júri

Presidente do Júri: *Prof. Doutora Inês Crespo*

Arguente: *Doutor Nuno Santos*

Orientador: *Prof. Doutora Teresa Mateus*

Orientador Interno

Prof. Doutora Teresa Mateus

Orientador Externo

Dr. Ricardo Brandão

(Cervas)

Dissertação do Estágio Curricular do Ciclo de Estudos Conducente ao Grau de Mestre em Medicina Veterinária da EUVG

Resumo

Nos dias de hoje, principalmente devido a fatores antropogénicos, existem mudanças ecológicas que permitem uma maior proximidade entre os animais selvagens e as áreas urbanas, o que leva a um maior contacto entre animais domésticos, animais selvagens e o Homem. A ocorrência de doenças zoonóticas, nomeadamente parasitárias, tendo os animais selvagens como hospedeiros é um problema de saúde pública disseminado pelo mundo. O conceito "One Health" veio sublinhar a ideia de que só dando igual importância à saúde animal, ambiental e humana é que é possível criar um ecossistema equilibrado e holisticamente saudável. Assim, os objetivos do presente estudo foram: avaliar a prevalência e a carga parasitária da helmintofauna gastrointestinal e avaliar a presença de *Trichinella* spp. em carnívoros selvagens de Portugal. Para este fim recolhemos amostras fecais e musculares de 16 texugos euroasiáticos, 10 ginetas e 9 fuínhas e apenas amostras fecais de 35 raposas num centro de recuperação de animais selvagens. As amostras fecais foram analisadas através do método Mini-FLOTAC e as musculares através do método de digestão artificial. Este será o primeiro estudo com carnívoros selvagens usando o método Mini-FLOTAC. Em 40 das 70 amostras fecais recolhidas (57,1%) foram identificadas formas parasitárias. As formas parasitárias mais prevalentes foram os ancilostomatídeos (Ancylostomatidae) (24,2%), seguidos dos géneros *Capillaria* (22,8%) e *Toxocara* (22,8%), tendo também sido identificados ovos das famílias Spiruridae, Oxyuridae e Taeniidae e as espécies *Toxascaris leonina* e *Alaria alata. Capillaria* spp. e os texugos alcançaram a maior carga parasitária. Não se identificaram larvas de *Trichinella* spp. Não se verificou relação entre a carga parasitária e a idade, o sexo ou a espécie dos animais. O estudo mostrou que em Portugal, à semelhança de outros países da Europa, ancilostomatídeos e *Toxocara* spp. são os agentes parasitários mais frequentes nestas espécies, o que, sendo estes potencialmente zoonóticos, pode constituir um perigo para a saúde pública, uma vez que a sua prevalência é muito elevada em animais sinantrópicos como a raposa. No entanto, estudos parasitológicos mais representativos devem ser realizados para que seja possível retirar mais conclusões acerca da situação atual, principalmente no caso de *Trichinella* spp..

Palavras-chave: *Alaria alata*, Ancylostomatidae, *Capillaria* spp., carnívoros selvagens, Spiruridae, Taeniidae, *Toxocara* spp., *Trichinella* spp..

Índice

Indice de tabelas

Lista de abreviaturas

CERVAS Centro de Ecologia, Recuperação e Vigilância de Animais Selvagens

CFSPH *Centre for Food Security and Public Health*

EPG *Eggs Per Gram*

FDT *FLOTAC dual technique*

g *Gram*

MF Mini-FLOTAC

ml *Millilitre*

WHO *World Health Organization*

º *Degrees*

% *Percent*

< *Less than*

Este manuscrito foi formatado de acordo com as regras da revista *Veterinary Parasitology* à qual será submetido

A survey of gastrointestinal parasites and *Trichinella* spp. in wild carnivores of Portugal

Ana Isabel Pereira Martins[a*], Ricardo Brandão[b**], Teresa Letra Mateus[a***]

[a] Departamento de Medicina Veterinária, Escola Universitária Vasco da Gama, Av. José R. Sousa Fernandes 197, Campus Universitário- Bloco B, Lordemão, 3020-210, Coimbra, Portugal
[b] Centro de Ecologia, Recuperação e Vigilância de Animais Selvagens (CERVAS), Associação ALDEIA, Apartado 126, 6290-909 Gouveia, Portugal.

* ana.pmartins29@gmail.com

** cervas.pnse@gmail.com

*** tlmateus@gmail.com

Abstract

Currently, mainly due to anthropogenic factors, there are ecological changes that allow a greater proximity of the wildlife to urban areas, increasing the contact between domestic animals, wild animals and humans. At the same time, the occurrence of zoonosis having wild animals as hosts is a major public health problem, affecting the whole world. The concept of the One Health underlined the idea that it is only by giving equal importance to animal, environmental and human health that a balanced and holistically healthy ecosystem can be achieved. Thus, the aim of the present study was to assess the prevalence and the burden of gastrointestinal helminth fauna and the presence of *Trichinella* spp. in wild carnivores from Portugal. For this purpose, faecal and muscular samples of 16 eurasian badgers, 10 genets, 9 stone martens and only faecal samples of 35 foxes were collected in a wildlife rescue centre. The faecal samples were analysed using Mini-Flotac method and the muscles by artificial digestion method. Of the 70 faecal samples in 40 (57.1%) gastrointestinal parasites have been found. The most frequent parasites were those of the family Ancylostomatidae (24.2%), followed by *Capillaria* spp. (22.8%) and *Toxocara* spp. (22.8%). Parasites of the families Spiruridae, Oxyuridae and Taeniidae and the species *Toxascaris leonina* and *Alaria alata* were also found. *Capillaria* spp. and badgers reached the higher burden. *Trichinella* spp. was not identified in any pool sample. It was found that neither the species, nor the age, nor the gender of the animals influenced the burden. The study showed that, in Portugal as in other European countries, hookworms and *Toxocara* spp. are the main danger to animal and public health, since their prevalence is very high in synanthropic animals, like foxes. *Trichinella* spp. has not been identified. Nevertheless, more representative parasitological studies should be carried out in order to draw more conclusions about the current situation, especially with respect to *Trichinella* spp..

Keywords: *Alaria alata*, Ancylostomatidae, *Capillaria* spp., Spiruridae, Taeniidae, *Toxocara* spp., *Trichinella* spp., wild carnivores.

Highlights:

- Mini-FLOTAC used for the first time for diagnosis in wild carnivores
- Parasites identified in 57.1% of the analysed samples
- Identification of several zoonotic parasites in synantropic carnivores

1. Introduction

Over the last few decades, natural factors like the expansion of wildlife population, and anthropogenic factors like deforestation, pollution and climate change have been occurring (Kruse et al., 2004). As a consequence, there are ecological changes that allow a greater proximity of the wildlife to urban areas, increasing the contact between domestic animals, wild animals and humans. About 62% of the agents considered to be pathogenic to humans are zoonotic (Taylor et al., 2001). Although domestic animals were historically considered as potential sources of zoonosis, nowadays, the occurrence of these diseases having wild animals as hosts is a major public health problem, affecting the whole world (Kruse et al., 2004; Cerbo et al., 2008). Particularly, wild carnivores are reservoir to a great variety of protozoans and helminths that could be transmitted to both domestic animals and humans (Cerbo et al., 2008).

Toxocariasis, for example, is a worldwide occurring zoonotic disease which presents a high prevalence in domestic and wild canids and felids, especially in tropical and sub-tropical countries (Okulewicz et al., 2012; Kuenzli et al., 2016).

Infections with tapeworms from the family Taeniidae are also potentially zoonotic diseases (Lymbery, 2017) . Among the 32 *Taenia* species known, only *Taenia multiceps* and *Taenia hydatigena* can affect wild carnivores and are potentially zoonotic (CSFPH, 2005). Despite the fact that they have no zoonotic interest, *T. pisiformis*, *T. polyacantha* and *T. serialis* are also important from the veterinary point of view, because they can infect foxes and other wild canids in addition to the domestic dog (Samuel et al., 2001). There is also a new genera proposed for this family of cestodes, *Versteria*, including the species *V. mustelae* and *V. brachyacantha* which have as final host mustelids (Lee et al., 2016). At each moment, more than 1 million people are affected by echinococcosis, making this disease a major public health problem throughout the world (WHO, 2017). *Echinococcus multilocularis*, which causes human alveolar echinococcosis, is well distributed throughout the northern hemisphere, including central Europe and the success of rabies vaccination programs implemented in foxes has contributed to the expansion of this cestode, because this carnivore is the most important final host for the parasite (Bagrade et al., 2008; Brossard et al., 2007). About *E. granulosus* the information is scarce, but the sylvatic cycle of this parasite has already been described in Portugal, by Guerra et al. (2013).

Trichinella spp. is considered one of the most widespread muscular parasites between carnivorous and omnivorous (Hurníková et al., 2007; Pozio, 2018). Although not of much interest from the veterinary perspective, trichinellosis is of great importance from a zoonotic point of view, since humans can function as a final host through ingestion of raw or undercooked meat from infected food animals or game meat (OIE, 2012). *Trichinella* spp. is one

of the many emerging zoonotic parasites that represent a major risk to the food animal production industry in general and to the consumer's health.

Furthermore, parasitic diseases can cause weight loss, diarrhoea, colic, apathy (Sprenger et al., 2018) anorexia, performance decrease, reproduction problems (Ilie et al., 2015), alterations in the central nervous system, respiratory, genitourinary, musculoskeletal lesions (Lempp et al., 2017) and other problems that are important for the conservation and management of wild species (Gortazar et al., 2007).These are the main reasons behind the increasingly importance of parasitological studies in wildlife.

Thus, the aim of the present study is to assess the prevalence and the burden of gastrointestinal helminth fauna and the presence of *Trichinella* spp. in wild carnivores from Portugal.

2. Materials and methods

2.1 Study area

The samples collected in this study come from four districts of Portugal: Guarda, Viseu, Bragança and Coimbra and they have been delivered to the centre of ecology, recovery and surveillance of wild animals (CERVAS). The centre is located in the natural park of Serra da Estrela. Serra da Estrela is part of the Natura 2000 network which works as a network of core breeding and resting sites, through which the most valuable and threatened species and habitats of Europe, both in land and in marine territory, are protected (European Commission, 2017).

2.2 Samples collection

The samples of faeces and muscle were collected during the necropsy of animals that were received at the centre between 2012 and 2018. The individuals arrived at centre alive or already dead.

2.3 Coprological method

The method used to evaluate the prevalence and burden of gastrointestinal parasites in faeces was Mini-FLOTAC (MF). This method allows the realization of a qualitative and quantitative analyzes due to the ruler grid of the chambers, which enables the counting of oocysts, larvae, cysts and eggs. To perform the flotation technique, one pipette, one rod, one plastic cup, one 50 ml beaker, one strainer, 2 g of fresh faeces and 18 ml of saturated sodium chloride solution were required. First, the sample and the saline solution were homogenized and filtered. Then, with the help of the pipette and using the filling holes, the flotation chambers were filled with the

faecal suspension until a little meniscus is formed. For the other chamber the same procedure was performed, but instead of the saturated sodium chloride solution, 1.350 zinc sulphate was used as the flotation solution. To the observation, the key was used to turn the reading disc clockwise (about 90°) until the reading disc stops moving and the microscope adaptor was attached to the microscope. Then the MF was placed on the microscope adaptor and we proceed to the observation. The observation was made with a magnification of 40x and the analytic sensitivity and multiplication factor of the test equals five eggs/oocysts/cysts/larvae per gram (EPG) of faeces (Anonymous, 2017).

2.4 Artificial digestion method

To assess the presence of *Trichinella* spp., the method of artificial digestion of pool samples was carried out. The technique was applied in diaphragm muscle. We started by dividing the samples into three pools, joining in the same pool the diaphragms of the same animal species. The pool of the genets, for example, contained four diaphragms which made up a total of approximately 15 g of muscle. Then, the meat was cleaned, by removing all the fat and connective tissue, and was crushed. To create the ideal conditions for the occurrence of artificial digestion, one liter of water preheated at 46-48 °C was put in a container to which was added 8 ml of hydrochloric acid and 5g of pepsin. The container was placed on top of a magnetic stirrer, it was covered with aluminium foil and we waited for 30 minutes, until the meat particles disappear. The suspension was then filtered and put in a separating funnel where it remained another 30 minutes. Next, 40 ml of the suspension was transferred to a graduated beaker and we waited 10 minutes. At the end, we discarded 30 ml of the supernatant and left 10 ml to observe in the magnifying glass. The observation was made with a magnification of 15 to 20x and, in the case of suspect areas, we used the 60 or 100x. This procedure was repeated for the eurasian badger and stone marten pools containing three and six muscle samples, respectively (Regulation (CE) nº 2075/2005).

2.5 Statistical analysis

The results were statistically analysed with the SAS program. To calculate the frequency and percentage of classes studied (species, gender and age) and the prevalence of each parasite in the study we used the FREQ procedure. To compare the burden by species, gender and age it was used the ANOVA one-way procedure.

3. Results

3.1. Characterization of the sample

Of the 70 animals, 45 entered the centre due to run over; two entered because of illness; one due to intoxication or poisoning; two due to weakness or malnutrition; one due to trauma and

one due to burns. The other 18 animals the cause that led to the entrance into the centre is unknown. Data collected to characterize the sample is presented in Table 1.

Table 1 – Number, gender and age by animal species

	Age			**Gender**			**Total**
	Juvenile	**Adult**	**Unknown**	**Male**	**Female**	**Unknown**	
Vulpes vulpes	6	20	9	18	3	14	35
Meles meles	0	12	4	7	1	8	16
Genetta genetta	0	7	3	3	1	6	10
Martes foina	0	5	4	3		6	9

Half of the samples belong to one animal species (*Vulpes* vulpes) and the remaining half distributed by the remaining three species of carnivores. About the sex, it was unknown for almost half (47.1%) of the sample. In relation to age, most (62.9%) of the animals were adults and all juveniles were foxes.

3.2. Faecal qualitative analysis – prevalence and diversity of parasites

A total of 70 faecal samples were collected and analysed and in 40 (57.1%) parasitic forms have been found (Table 2). On the whole, three families, two genera and three species of parasites were recorded.

Table 2 – Frequency and prevalence (%) of parasites identified by animal species

Parasite	*Vulpes vulpes*	n/N	Confidence interval (95%)	*Meles meles*	n/N	Confidence interval (95%)	Overall Prevalence
Ancylostomatidae	37.1%	13/35	0.21 - 0.52	12.5%	2/16	0.00 - 0.29	24.2%
***Toxocara* spp.**	31.4%	11/35	0.16 - 0.47	12.5%	2/16	0.00 - 0.29	22.8%
***Capillaria* spp.**	22.8%	8/35	0.09 - 0.37	18.7%	3/16	0.00 - 0.38	22.8%
Spiruridae	11.4%	4/35	0.01 - 0.22	6.2%	1/16	0.00 - 0.18	7.1%
Toxascaris leonina	5.7%	2/35	0.00 - 0.13	n.i.	-	-	2.8%
Taeniidae	5.7%	2/35	0.00 - 0.13	n.i.	-	-	4.2%
Oxyuridae	n.i.	-	-	6.2%	1/16	0.00 - 0.18	2.8%
Alaria alata	2.8%	1/35	0.00 - 0.08	n.i.	-	-	1.4%

(n.i. – not identified)

Table 2 (Cont.) – Frequency and prevalence (%) of parasites identified by animal species

Parasite	*Martes foina*	n/N	Confidence interval (95%)	*Genetta genetta*	n/N	Confidence interval (95%)	Overall prevalence
Ancylostomatidae	n.i.	-	-	20.0%	2/10	0.00 - 0.45	24.2%
***Toxocara* spp.**	n.i.	-	-	30.0%	3/10	0.02 - 0.58	22.8%
***Capillaria* spp.**	44.4%	4/9	0.12 - 0.77	n.i.	-	-	22.8%
Spiruridae	n.i.	-	-	n.i.	-	-	7.1%
Toxascaris leonina	n.i.	-	-	n.i.	-	-	2.8%
Taeniidae	11.1%	1/9	0.00 - 0.32	n.i.	-	-	4.2%
Oxyuridae	11.1%	1/9	-	n.i.	-	-	2.8%
Alaria alata	n.i.	-	-	n.i.	-	-	1.4%

(n.i. – not identified)

Nematodes were identified in all (100%) of the 35 samples from red foxes, in 43.8% of the badgers and in 40.0% of the genets. Cestodes were found only in red foxes and stones martens and trematodes only in faeces of red foxes.

The higher diversity of parasitic forms was found in red foxes (n=7). In euroasian badger it was found five different species. In stone martens three different parasites have been found and in genets two.

The animals were parasitized mostly with one parasitic form (24/70, 34.2%), but in some samples two (13/70, 18.5%), three (3/70, 4.2%) and five (1/70, 1.4%) different parasitic forms were identified.

Hookworms were found with the highest prevalence (24.2%) in this survey. *Capillaria* spp. was identified in 22.8% of the samples, presenting the same prevalence as the ascarid *Toxocara* spp. (22.8%). The only spirurid found and *Toxascaris leonina* had a prevalence of 7.1% and 2.8%, respectively, and oxyurids were also found in 2.8% of the samples. Regarding cestodes, Taeniidae have been found with a prevalence of 4.2%. The only trematode found in this study was *Alaria alata,* with a prevalence of 1.4%. *Alaria alata* and *Toxascaris leonina* have been identified only in red foxes.

3.3 Faecal quantitative analysis

Table 3 presents the burden of eggs per gram off faeces of parasites found by parasite.

Table 3 - Mean, standard deviation and maximum number of EPG of faeces by parasite

Parasite	**Number of samples**	**Mean**	**Std Dev**	**Maximum**
***Capillaria* spp.**	15	94.86	716.60	6000
***Toxocara* spp.**	16	59.79	333.52	2660
Toxascaris leonina	2	9.71	72.74	605
Taeniidae	3	7.79	58.73	490
Ancylostomatidae	17	11.29	33.72	215
Alaria alata	1	0.50	4.18	35
Spiruridae	5	0.79	3.47	25
Oxyuridae	2	0.43	3.04	25

Considering the burden, there were a few remarkable values: the highest burden found was 6000 EPG of faeces (*Capillaria* spp.) in a sample of a badger and 2660 EPG of faeces (*Toxocara* spp.) in a sample of a red fox. Concerning cestodes, the burden of one red fox sample was much higher (490 EPG) than the other two samples. The only trematode of the study, *Alaria alata*, was found with a burden of 35 EPG of faeces.

It was found that neither the species, nor the age, nor the gender of the animals influenced the number of EPG of faeces present in the samples (P-value > 0.05).

3.4 Analysis of Trichinella spp.

Regarding the research of *Trichinella* spp., in the three pools of samples of badgers, genets and stone martens this parasite was not identified.

4. Discussion

In most European countries researches have been carried out to study the helminth fauna of the red fox and other carnivores. Foxes are very abundant and widespread in the whole world, but particularly in western and central Europe, it occurs due to factors such as the implementation of anti-rabies control programs (Deplazes et al., 2004), and its approximation to the human population (Wandeler et al., 2003). This synantropic lifestyle allows it to benefit from conditions that are favourable to the maintenance of the species, such as easy access to food. This carnivore, depending on the geographic area, the habitat in which they are found and the prey availability, can be host for a wide variety of helminths and protozoan parasites (Fiocchi et al., 2016). It is widespread and abundant in the Central region of Portugal (Figueiredo et al., 2016).

In the present research, Ancylostomatidae and *Toxocara* spp. were classified as the main species in red foxes. Although we do not know the genus or species of the hookworm, *Uncinaria stenocephala*, is one of the most prevalent in many other studies on foxes in Europe (Deblock et al., 1988; Suchentrunk & Sattmann, 1994; Willingham et al., 1996; Smith et al., 2003; Segovia et al., 2004; Rataj et al., 2013), and also in the central coast of Portugal (Eira et al., 2006). The Ancylostomatidae prevalence in red foxes ranges between 41.3% and 58.9% in countries like Great-Britain, Greece and Slovenia (Papadopoulos et al., 1997; Smith et al., 2003; Rataj et al., 2013). Ancylostomatidae are parasites of the small intestine of domestic and wild carnivores, and are important also because of their zoonotic potential. In 2010, the number of humans infected by hookworms was around 440 million people (Pullan et al., 2014). This disease primarily affects rural communities in tropical and subtropical countries (Hotez et al., 2004). The most important species of hookworms for wild carnivores are *Uncinaria stenocephala, Ancylostoma caninum, A. braziliense* and *A. ceylanicum*; (Campillo et al., 1999; Taylor et al., 2007). In Spain, Miquel et al. (1994) found *U. stenocephala* in foxes with a high prevalence (78.46%) and also found *A. caninum* but with a much lower prevalence (1.54%). In Portugal, Figueiredo et al. (2016), Guerra (2012) and Silva (2010) identified the presence of Ancylostomatidae in foxes with a prevalence of 10.71%, 43.5% and 64.2%, respectively. Also in red foxes, Eira et al. (2006) found the species *U. stenocephala* with a prevalence of 77.4%. In the central region of Portugal, the presence of stone martens parasitized with Ancylostomatidae was observed, reaching a prevalence of 8% (Figueiredo et al., 2018).

Dogs, particularly those of farms, hunting or free roaming, can share the same environment as the wild carnivores, allowing the transmission of disease agents like parasites. In a study carried out with samples of these groups of dogs collected in Ponte de Lima, Portugal, parasites of the family Ancylostomatidae were detected with the highest prevalence, followed by *Trichuris* spp.

and *Toxocara* spp., which are also potentially zoonotic (Mateus et al., 2014). In another study developed in Portugal with soil samples and dog faeces collected in 10 public parks in Coimbra (a city in the central region of Portugal), it was found that five parks were contaminated with parasite forms of the family Ancylostomatidae and *Toxocara* spp., which proves that pets living in urban centres are also infected, in particular with these two nematodes (Cruz et al., 2012). Thus, intervening in the transmission of these hookworms between the domestic and wild animals has a crucial role in preventing the disease also in humans.

Although dogs and cats are the most frequent hosts of *Toxocara* spp., wild canids such as jackals, hyenas, foxes and wolves are also important reservoirs for these species. There are 27 species of the genus *Toxocara* and three of these are responsible for toxocariasis in humans, due to the ingestion of infective eggs: *T. canis, T. cati* and *T. pteropodis* (Borecka, 2010). These parasites can have different clinical manifestations in humans which result from the migration of the hatched larvae in organs like liver, heart, lungs, eyes, muscle and brain (Magnaval et al., 2001). Environmental contamination due to the presence of faeces of cats and dogs in urban and rural areas acts as the main focus of infection to humans, domestic animals (Guerra et al., 2012) and wild animals as well. Furthermore, foxes in particular, play an important role in the infection of dogs by *T. canis* (Duscher et al., 2015). There are several studies referring to the presence of eggs of *Toxocara* spp. in foxes, particularly of *T. canis*. In Europe, *T. canis* was frequently found in faecal samples of foxes from Great-Britain (61.1%) (Smith et al., 2003), Spain (30.0%) (Miquel et al., 1994) Slovenia (38.3%) (Rataj et al., 2013), Greece (28.3%) (Papdopoulos et al., 1997) and with lower prevalence in Switzerland (14.0%) (Okulewicz et al., 2012). In our study, this ascarid was the one with the highest parasitic burden (2660 EPG of faeces) and this fox had died of unknown disease, maybe the cause has been toxocariosis. Eira et al. (2006), Guerra et al. (2012) and Silva et al. (2012) described these parasites with high prevalence in red foxes from Portugal too (15- 40%, 24.7% and 12.3%, respectively).

While predators, genets are also frequently targeted by helminthic parasites. From the 15 species of the genus *Toxocara*, three had already been found in faecal samples of genets: *T. cati* (Cordero del Campillo, 1980)*, T. vincenti* (Warren, 1972) and *T. genettae* (Warren, 1972; Alvarez et al., 1990). For the first time in Europe, Alvarez et al. (1990) found *T. genettae* in small intestine of 73% spanish genets and in another study also performed in Spain, this nematode also occurs with high prevalence (75%) (Sanmatín et al., 1992). In the present study, it was found that 3 out of ten genets were parasitized with *Toxocara* spp. and 2 with Ancylostomatidae. In relation to hookworms, Colon & Patton (2012) refered that *Ancylostoma* spp. is a nematode that is commonly found in small carnivores like genets. The presence of *Ancylostoma martinezi* has already been verified in Spain, with a prevalence of 8.33% (Miquel et al., 1994). Both *Toxocara* spp. and Ancylostomatidae eggs had already been reported in genets from Northern Portugal (Mateus & Barrocas, 2012) as well.

Toxascaris leonina is also a parasite of canids and felids such as dogs, cats and foxes, although it is less frequent than other ascarids of carnivores (Cordero del Campillo, 1999). This parasite, generally, has a monoxenous life cycle, however rodents can play the role of optional intermediate hosts (Okulewicz et al., 2012; Monsonís, 2015). In Europe, this was documented in Spain (25.17%) by Monsonís (2015) and by Miquel et al. (1994) (30.0%), in Switzerland (8.0%) (Reperant et al., 2007), in Italy (5.4%) (Magi et al., 2009), in Slovenia (2.5%) (Rataj et al., 2013), in United Kingdom (1.5%) (Richards et al., 1995) and in Denmark (0.6%) (Saeed et al., 2006). In Portugal, Silva (2010) and Figueiredo et al (2016) also verified the presence of the parasite in foxes, presenting a prevalence of 1.2% and 10.71%, respectively. Usually, *Toxascaris leonina* is accompanied by *Toxocara* spp. infection in both domestic and wild canids (Taylor et al., 2007; Okulewicz et al., 2012), as it can be seen in our study.

The most common species of Spiruridae family occurring in domestic and wild canids is *Spirocerca lupi*, although it has also been described in mustelids and viverrids and it can, occasionally, be found in cats and wild felids too (Perez & Palma, 2001; Taylor et al., 2007). This parasite has as paratenic hosts coprophagous beetles, like *Scarabeus sacer, Akis, Atenchus, Gymnopleurus*, *Cauthon* spp. and many vertebrates such as rodents, birds, insectivores and reptiles (Taylor et al., 2007). Spirocercosis is characterised by the occurrence of pathognomonic lesions such as nodular formations in oesophagus, stomach and aorta (Cordero del Campillo et al., 1999). The disease has a wide distribution in tropical and sub-tropical regions, and it's also relatively frequent in temperate zones (Cordero del Campillo et al., 1999; Taylor et al., 2007). The incidence of infection in dogs from tropical and sub-tropical regions is often extremely high, sometimes approaching 100%, but although it's important from the veterinary point of view, the parasite has no zoonotic risk (Taylor et al., 2007). This nematode has been described mainly in foxes from some European countries, such as Serbia (Pavlović et al., 1997), Spain (Gortázar et al., 1998; Segovia, 2004), Denmark (Al-Sabi et al., 2014), Greece (Diakou et al., 2012) and Italy (Ferrantelli et al., 2010). A very similar prevalence in foxes (12.9%) with the present study was found in Portugal by Eira et al. (2006). In this present study, four foxes and one eurasian badger were parasitized by a nematode of this family, maybe *Spirocerca lupi* since, particularly in Europe, there is a strict relation between spirocercosis and wildlife and because it is considered to be very frequent (Giannelli et al., 2014). On the other hand, in Spain, another parasite of the same family is described in badgers, the species *Vigisospirura potekhina hugoti* (Torres et al., 1997). The individual parasitized with this species in the spanish survey was found in the province of Cáceres, which borders Portugal near the region where our samples were collected, so the question remains open.

Capillaria spp. is also a well-known potentially zoonotic nematode occurring in dogs and many other animals. The most important species for humans are *C. aerophila, C. hepatica* and *C. philippinensis.* Molecular assays have shown that *C. aerophila* populations are shared between wild and domestic carnivores from various countries of Europe, and red foxes and stone

martens may contribute to the spreading of the parasite in areas where, previously it was not endemic (Di Cesare et al., 2012). Animals infected with *C. aerophila* may show subclinical effects or may develop respiratory problems wich can go from mild to severe and potentially fatal pneumonia (Otranto et al., 2015). Although it's rare, occasionally humans can act as definitive hosts as well (Lalošević et al., 2008). Furthermore, it is believed to be a cosmopolitan parasite, which further underlines its importance for public health (Di Cesare et al., 2012). In Europe, it is found with high prevalence in foxes from Norway (88%) (Davidson et al., 2006), Denmark (74.1%) (Saeed et al., 2006), Hungary (66%) (Sréter et al., 2006) and Netherlands (46.8%) (Borgsteede, 1984).

The hosts of *C. hepatica* are rats and this is the reason why the parasite have such a wide distribution globally and it explains why it is a major public health problem (Zamini et al., 2017). The route of infection to humans is through ingestion of soil, water or animal carcasses containing embryonated eggs (Belizario and Totañes, 2014). We have found this nematode with high prevalence (44.4%) in stone martens, which suggests the idea that these animals are an important reservoir for the parasite in Portugal. *Capillaria* spp. have also been described in stone martens from Poland (Kornas et al., 2013), Austria (Visser et al., 2011), Germany (Schoo et al., 1994) and Italy (Millán & Ferroglio, 2001; Balestrieri et al., 2006). In Portuguese foxes, *Capillaria aerophila* has been found by Silva (2010) with low prevalence (3.7%) and in our study, the genus *Capillaria* was found with a higher prevalence (22.9%). Many other European countries have observed the presence of the parasite in these animals, namely Denmark (36.8%) (Willingham et al., 1996), Ireland (Wolfe et al., 2001), Serbia (Ilić et al., 2016), Italy (7%) (Magi et al., 2009) and Norway (*C. boehmi* – 51%; *C. plica* – 53%; *C. aerophila* – 88%) (Davidson et al., 2006). The presence of *Capillaria* in badgers has already been described also in England, (15.9%) (Cottrell, 2011) and in Italy, with a prevalence relatively higher (31.6%).

In the present study, Taeniidae eggs were reported in two red foxes and 1 stone marten. Some taeniid tapeworms "…are historical ecological indicators of the foraging behaviour and food habits of our early ancestors during the diversification of *Homo sapiens*, or humans" which explains the zoonotic potential of these parasites (Hoberg, 2001). Although some species are widely distributed and can cause enormous public health problems, since they can infect humans, little is known about their epidemiology in wildlife, mainly because of the difficulty in developing surveys in these animals (Schneider-Crease et al., 2017).

As happened in the present study, wild canids such as foxes can function as hosts for a wide variety of parasite species belonging to the Taeniidae family. *Taenia multiceps* and *T. serialis* are parasites with wide distribution in temperate areas and their larval stage cause coenurosis, a zoonotic disease affecting the subcutaneous tissues, muscles, and central nervous system of intermediate hosts or humans (Webman & Gilman, 2013). The adult form of *T. multiceps* is described in domestic and stray dogs, wolves, coyotes, jackals and rarely in felids, but there's a gap in knowledge about the epidemiology of this parasite in foxes (Varcasia et al., 2015). In

Europe, the parasite has only been identified in foxes from Italy (Varcasia et al., 2015) and Romania (Barabási et al., 2010) and it´s described with low prevalence (6.3% e 4.6%, respectively). Although the epizootiology of *T. serialis* it's not very well known, the parasite has also been described in foxes and jackals from countries like Romania (Barabási et al., 2010), Great-Britain (Jones & Walters, 1992) and Germany (Loos-Frank & Zeyhle, 1982; Samuel et al., 2001.

Cysticercosis is also a zoonotic disease caused by many species of *Taenia*, namely *T. solium, T. saginata, T. crassiceps, T. ovis, T. taeniaeformis, T. hydatigena*, although it occurs most frequently with *T. solium* (CSFPH, 2005). *T. hydatigena* has a wide distribution in carnivores like the domestic dog and wild canids, such as red foxes, coyotes, jackals and wolves (Samuel et al., 2001). In red foxes, the parasite is described in countries like Italy (Fiocchi et al., 2016), Germany (Loos-Frank & Zeyhle, 1982; Pfeiffer et al., 1997), United Kingdom (Richards et al., 1995), Romania (Ilie et al., 2015) and Slovak Republic (Letková et al., 2008) In the 1970s it was thought that foxes were rarely infected by *Taenia pisiformis*, due to an age resistance that would be independent of previous exposure to the parasite, but more recently it is known that this animal can also be definitive host of the parasite (Pritt et al., 2012). It has been described in red foxes from United Kingdom (Richards et al., 1995), Great-Britain (Jones & Walters, 1992), Romania (Ilie et al., 2015) and Slovenia (Rataj et al., 2013). Other wild carnivores like wolves, coyotes and felids, like the wild cat, has also been reported with the parasite in various countries of Europe (Samuel at al., 2001).*Taenia polyacantha* is described in arctic foxes from the tundra zone in Alaska (Rausch & Fay, 1988) and Canada (Webster,1974); and in red foxes from the southern region of the Northern Hemisphere, in countries like Austria (Hinaidy, 1971), Slovenia (Rataj et al., 2013), Germany (Pfeifer, 1996), Slovak Republic (Létková et al., 2008), Romania (Barabási et al., 2010), France (Deblock et al., 2011) and Great Britain (Jones & Walters, 1992). In Portugal the species *T. hydatigena* e *T. polyacantha* have already been described by Guerra (2012).

Another genus belonging to the Taeniidae family is *Echinococcus*. *E. multilocularis* and *E. granulosus* are cestodes with a major importance due to its zoonotic potential, causing alveolar echinococcosis and hydatid disease, respectively. The expansion of the fox population due to anti-rabies vaccination has been increasing the prevalence of *E. multilocularis*, which translates into an increased pressure of infection with the parasite in intermediate and accidental hosts such as humans (Gottstein et al., 2015). This disease has severe clinical implications in humans and, normally, occurs with high burdens (Otranto et al., 2015). It is known that in Europe, the main definitive host of *E. multilocularis* is the red fox, although the domestic dog and cat, and other wild carnivores such as coyotes, raccoons, wolves or wild cats can also play this role (Conraths & Deplazes, 2015). Although dogs carry a low prevalence of this parasite, they function as important agents of environmental contamination (4-19%) (Hegglin and Deplazes, 2013). *E. multilocularis* has been described with medium prevalence in foxes from Slovenia (2.6%) (Rataj et al., 2013) and Italy (9.6%) (Casulli et al., 2005) and with higher prevalence in

countries like Latvia (35.6%) (Bagrade et al., 2016), Hungary (29.4%) (Sréter et al., 2003) and many other from central and eastern Europe (Oksanen et al., 2016).
In countries like Spain and Portugal, *E. granulosus* only occurs in sporadic cases in humans (Otranto et al., 2015). Although in Europe *E. granulosus* mainly uses domestic dogs as definitive hosts and domestic ungulates as intermediate hosts, it has already been described in foxes from various countries, such as Finland (Hirvelä-Koskiet al., 2003), Bulgaria (Breyer et al., 2004), Italy (Guberti et al., 2004) and Spain (Sobrino et al., 2006), in addition to Portugal (Guerra et al., 2013). Usually this animal harbors a low burden of the parasite, not being considerate an important agent for its transmission (Otranto et al., 2015).
In this study it was also identified a parasite of this family in a stone marten. Mustelids have been reported with species like *T. martis* and *T. mustelae* (Samuel et al., 2001).

In the past, the lack of information about the biology of *Alaria alata* led to confusion regarding the risk of human exposure to the parasite, and the humans appear to be a negligible and accidental participant in the cycle (Möhl et al., 2009). However, today, this issue has been a cause for concern due to the recently found of mesocercariae in meat of wild boars, during official *Trichinella* spp. inspection (Möhl et al., 2009; EFSA, 2016). In Europe, the adult form of the parasite was found in red foxes (Murphy et al., 2012), racoon dogs (*Nyctereutes procyonoides*) (Rentería-Solís et al., 2013), wolves (*Canis lupus lupus*) (Hermosilla et al., 2017), badgers (Takeuchi-Storm et al., 2015) and domestic cats (*Felis catus*) (Takeuchi-Storm et al., 2015). In the present study, we only found it in one red fox. The low prevalence of this parasite might be due to the fact that the study area is a mountainous region and does not contain a large percentage of surface water, since the intermediate host is an aquatic snail. However, Segovia et al. (2004) also verified its presence in Serra da Malcata, which is close to Serra da Estrela, with a higher prevalence, amounting to 19.2%. On the other hand, in another study conducted by Eira et al. (2006) also in Portugal, in the central coast of the country, the prevalence was higher (27.42%), maybe because in this region the environmental characteristics are more in agreement with those necessary for the survival of the intermediate host.

Eggs of oxyurids were found in faeces from one stone marten and one eurasian badger. No information has been found regarding oxyurids infection in any of the animals mentioned, which leads us to suspect that these eggs may be from the prey of these carnivores. Stone martens are opportunistic feeders, their diet varies depending on food supply and prey availability, feeding mainly on birds, small mammals (like rodents and lagomorphs), fruit and insects (Posluszny et al., 2007). Regarding the eurasian badger, its diet depends on the geographical area in which they live and it includes mammals, reptiles, birds, molluscs and annelids, but its main food source are vegetables and arthropods (Del Bove and Isotti, 2001). Free-ranging birds function as hosts for a wide variety of gastrointestinal parasites that can be transmitted to other animals and even to humans. In a study in England, oxyurids have already been described in

free ranging birds (Játiva, 2018). Pin worms are also very frequently found in reptiles (Rinaldi et al., 2012) On the other hand, these nematodes can also parasite invertebrates such as diplopods, coleopterans, crickets and other arthropods (Carreno, 2014), which can also explain the presence of the eggs in both samples.

As well as the prevalence of each parasite, it is also important to analyse the burden. In our study, *Capillaria* spp. and badger have the highest burden. In badgers with severe *Capillaria* spp. infections, especially in cubs, symptoms including diarrhoea, vomiting, and weight loss are very frequent, which may be due to the fact that they have a different lifestyle from the adults (they are completely fossorial until 8 weeks old and they live underground until 10 weeks), or due to their immune system, that is little developed (Cotrell, 2011). Just like the epidemiology, pathological effects and life cycle, also the parasitic burden that each animal presents is a complex subject, which is dependent on several factors, such as: age (Douche & Moram, 1993), feeding and other behaviours (Rosalino et al., 2006), gender (Poulin, 1996), cycles in season (Haukisalmi et al., 1988) and geographical range (Torres et al., 2001). However, in our study, it was found that there was no significant variability when comparing adults and juveniles, female and male animals, or between the different carnivorous species. This may be due to the absence of representativeness of the sample, ie, the fact that it is divided into groups with different dimensions, or because there were many animals whose gender and age were indeterminate which also influenced the results. Cotrell (2011) did a study in England, where it was found that *Capillaria* spp. burden in badger is significantly correlated with month of sampling (highest egg output in August), age (higher prevalence in cubs) and body condition index of the host (negative correlation between burden and body condition index). In the present study it is highlight the burden of Taeniidae because these parasites are potentially zoonotic and are immediately infective and can be spread by wind and rain, contaminating vegetation and water (CSFPH, 2005). Ancylostomatidae, *Toxocara* spp. and *Alaria alata* eggs are also important because of the environmental contamination, as they can also parasitize humans. The maximum number of *Toxocara* spp. eggs found was 2660 EPG of faeces. In a study developed in the north of Portugal (Silva, 2010) found that the burden of *T. canis* in foxes was higher, reaching 8000 EPG of faeces. Although we do not know the age of the population used in the study to which we refer, we can suggest that it is a population constituted by younger animals, which would explain the high burden value. Roddie et al. (2008) studied these parasite burden on foxes from Ireland, and found that the range was from 0 to 2352 EPG. However, Martínez-Carrasco et al. (2007) found that Spanish foxes presented a *Toxocara canis* burden reaching only 35 EPG of faeces. In the present study the burden of Ancylostomatidae on foxes is in the range of 0 – 215 EPG of faeces. When compared with the maximum burden described in the study of Silva (2010), we found that in the later the EPG value found is much higher, reaching 6200 EPG of faeces. This discrepancy in values may be justified by the fecal sampling method, since in the study of Silva (2010) the sampling was performed directly in the field with fresh

samples and, in the present study, the samples were collected from frozen cadavers, some of them for years.

Considering the quantitative analysis, to the authors knowledge, this is the first survey on wild carnivores done with MF. This has advantages over other techniques due to its versatility, since it allows the diagnosis of both larvae and eggs of helminths, as oocysts and cysts of protozoa. This method is an innovative copromicroscopic technique with high precision, accuracy and sensitivity in results (Cringoli et al., 2017). In a recent study using dog faeces' samples, the sensitivity of various copromicroscopic tests (direct smear, flotation in tube, MF, Wisconsin, FLOTAC dual technique) was compared and it was found that MF, as well as FLOTAC dual technique, is the most sensitive method and is therefore considered the standard test to diagnose the presence of gastrointestinal parasites in dog faeces (Maurelli et al., 2014). Thus, we can assume that this is also the best choice in the diagnosis of helminths in wild carnivores.

Trichinellosis is a well distributed disease which is transmitted by the ingestion of infected meat or meat products (OIE, 2018). Although four *Trichinella* species are known to circulate in Europe, only two are considered as major, *T. britovi* and *T. spiralis*. In countries like Germany, Spain, Finland and Poland, *T. spiralis* is the most prevalent, but in most other countries is *T. britovi* (Pozio et al., 2009). *T. spiralis* is primarily a trichinellosis agent in domestic animals, although it may also affect wildlife; contrary to *T. britovi* whose life cycle is only sylvatic (Magalhães et al., 2004). Although horses and boars can be infected, the main agents in perpetuating the sylvatic cycle are wild carnivores such as foxes and mustelids, among others; this is why the eradication of the disease becomes impossible (Magalhães et al., 2004; Pozio et al., 2009). Rats also play an important role in the transmission of the parasite, contributing also to the eradication difficulty (OIE, 2018). In Europe, the presence of *T. spiralis* is described in canids, such as red foxes, jackals and wolves; mustelids, such as stone martens and badgers; and in felids like lynx, domestic cats and wild cats (Pozio et al., 2009). In Portugal only the presence of *T. britovi* in wild carnivores is described, and occurred in only seven individuals (Pozio et al., 2009). In another study carried out in Portugal, was addressed the geographical distribution of the parasite; for that it was compared the prevalence in wild animals from the mountainous region with the one of the animals from lowlands and it was concluded that the probability of infection is much higher in mountain areas and natural parks in these regions than in lowland zones like Alentejo (Magalhães et al., 2004). In the present study, *Trichinella* spp. was not identified, however, it should be noted that red fox diaphragms have not been studied and this represents the main carrier of this parasite in the European Union (Hurnikova & Danica, 2007). Regarding the technique used for the diagnosis of *Trichinella* spp., Franssen et al. (2014) showed that the sequential sieving method is the fastest way for the detection of muscular larvae in frozen meat, nevertheless we used the magnetic stirrer artificial digestion technique, due to its effectiveness, since it is the European Union reference method for the surveillance of the *Trichinella* spp. infection in wildlife.

We underline the fact that the sample collection consists of samples of a reasonable number and an interesting diversity of wild carnivores. However, the animals come from dispersed areas, which does not allow to draw conclusions about the parasitic state of the animals of a more delimited region. In fact, more localized surveillance, through the analysis of faeces of wild carnivores living in rural areas surrounding the cities from where these animals come, could give more useful information, since it is in these areas that there is a high zoonotic risk.

The results of this study emphasized the importance of developing studies on the prevalence and parasitic diversity in wild fauna, mainly in synanthropic species, because of the risk they pose to the health of domestic animals and, consequently, to humans. The recent emergence of the One Health concept underlined the idea that it is only by giving equal importance to animal, environmental and human health that a balanced and holistically healthy ecosystem can be achieved. Only on a planet where there is complete symbiosis and health for all inhabitants is it possible to live a happy and prosperous life.

5. References

Al-Sabi, M. N. S., Hansen, M. S., Chriél, M., Holm, E., Larsen, G. & Enemark, H. L. (2014). Genetically distinct isolates of *Spirocerca* sp. from a naturally infected red fox (*Vulpes vulpes*) from Denmark, Veterinary Parasitology, 205(1–2), 389-396.,

Alvarez, F. R., Iglesias, J., Bos, J., Tojo, J., Sanmartim, M. L. (1990). New findings on the helminth fauna of the common European genet (*Genetta genetta L*.): first record of *Toxocara genettae* Warren, 1972 (Ascarididae) in Europe. Annales de Parasitologie Humaine et Comparé, 65, 244 – 248.

Anonymous (2017). Parassitologia veterinaria. Mini-FLOTAC. Retrieved from: http://www.parassitologia.unina.it/flotac/mini-flotac/

Bagrade G., Deksne G., Ozolina Z., Howlett S.J., Interisano M., Casulli A., Pozio E. (2016). *Echinococcus multilocularis* in foxes and raccoon dogs: an increasing concern for Baltic countries. Parasites & Vectors, 9 (1), 615.

Bagrade, G., Šnábel, V., Romig, T., Ozoli\cnš, J., Hüttner, M., & Miterpáková, M. (2008). *Echinococcus multilocularis* is a frequent parasite of red foxes (*Vulpes vulpes*) in Latvia. Helminthologia, 45.

Balestrieri, A., Remonti, L., Ferrari, N., Ferrari, A., Lo Valvo, T., Robetto, S., Orusa, R. (2006). Sarcoptic mange in wild carnivores and its co-ocurrence with parasitic helminths in the Western Italian Alps. European Journal of Wildlife Researh, 52(3), 196-201.

Barabási, S. S., Fok, E., Gubányi, A., Mészáros, F., Cozma, V. (2010). Helminth fauna of the small intestine in the European red fox, *Vulpes vulpes* with notes on the morphological identification o*f Echinococcus multiloculares.* Sci Parasitol 11(3), 141-151.

Belizario Jr. V. Y & Tonañes, F. I. G. (2014). Helminth-Nematode: *Capillaria hepatica* and *Capillaria philippinensis.* Enyclopedia of Food Safety, 2, 90-93.

Borecka, A. (2010). [The spread of nematodes from *Toxocara* genus in the world]. Wiadomosci parazytologiczne, *56*(2), 117–124.

Borgsteede, F. H. M. (1984). Helminth parasites of wild foxes *(Vulpes vulpes L.)* in The Netherlands. Z Parasitenkd*,* 70, 281-285.

Breyer, I., Georgieva, D., Kurdova, R., Gottstein, B. (2004). *Echinococcus granulosus* strain typing in Bulgaria: the G1 genotype is predominant in intermediate and definitive wild hosts. Parasitol. Res. 93, 127–130.

Brossard, M., Andreutti, C., & Siegenthaler, M. (2007). Infection of red foxes with *Echinococcus multilocularis* in western Switzerland. Journal of Helminthology, *81*(4), 369–376.

Carreno, R. A. (*2014*) The Systematics and Evolution of Pinworms (Nematoda: *Oxyurida:* Thelastomatoidea) from Invertebrates. Journal of Parasitology*,* 100 (5), 553-560.

Casulli, A., Manfredi, M. T., La Rosa, G., Di Cerbo, A. R., Dinkel, A., Romig, T., Pozio, E. (2005). *Echinococcus multilocularis* in red foxes *(Vulpes vulpes*) of the Italian Alpine region: is there a focus of autochthonous transmission? International Journal for Parasitology*,* 35 (10), 1079 –1083.

Cerbo, A., Manfredi, M., Bregoli, M., et al. (2008). Wild carnivores as source of zoonotic helminths in north-eastern Italy. Helminthologia, 45(1), pp. 13-19.

CFSPH (2005). Zoonotic diseases – Technical factsheets: Taenia infections. Retrived April 29, 2018 from: http://www.cfsph.iastate.edu/Factsheets/pdfs/taenia.pdf

Colon, C. P. and Patton, S. (2012). Parasites of Civets (Mammalia, Viverridae) in Sabah, Borneo: A Coprological Survey. Malayan Nature Journal. 64(2), 87-94.

Conraths, F. J., Deplazes, P. (2015). *Echinococcus multilocularis:* Epidemiology, surveillance and state-of-the-art diagnostics from a veterinary public health perspective. Vet Parasitol., 213(3-4): 149-161.

Cordero del Campillo, M. (1980). Indice – Catálogo de Zooparásitos Ibéricos. Servicio de publicaciones. Ministerio de Sanidade e Seguridade Social, Madrid, pp. 179.

Cordero del Capillo, M., Vazquez, F. A. R., Fernandez, A.R.M, Acedo, M. C. S., Rodriguez, S. H., Lopez-Cozar, I. N., Bañoz, P. D., Romero, H. Q., Varela, M. C. (1999). *Parasitologia veterinaria* (1st Ed.). Mc-Graw Hill Interamericana de España, S. A. U.

Cottrell, E. R. A. (2011). Variation of parasite burden within the European badger *(Meles meles):* the effect of season, habitat, body condition, gender & age on the prevalence of *Eimeria melis and Capillaria.* Thesis for the degree of Master of Science, University of Exeter, Exeter, Devonshire, England.

Cringoli, G., Maurelli, M. P., Levecke, B., Bosco, A., Vercruysse, J., Utzinger, J. & Rinaldi, L. (2017). The Mini-FLOTAC technique for the diagnosis of helminth and protozoan infections in humans and animals. Nat. Protoc. 5 , 503-515.

Cruz, A., Santos, A., Mateus, T., Ramalho, F., Sousa, S. & Madeira de Carvalho, L. (2012). Avaliação do grau de contaminação ambiental com formas parasitárias zoonóticas com origem em dejectos caninos em áreas públicas da cidade de Coimbra. Evaluation of the environmental contamination degree with zoonotic parasitic forms due to canine feces in public áreas of the city of Coimbra. Acta Parasitológica Portuguesa, 19, 35-38.

Davidson, R. K., Gjerde, B., Vikoren, T., Lillehaug, A., Handeland, K. (2006). Prevalence of Trichinella larvae and extra-intestinal nematodes in Norwegian red foxes *(Vulpes vulpes).* Veterinary Parasitology, *136*(3–4), 307–316.

Deblock, S. & F. Pétavy, A. & Gilot, B. *(2011).* Helminthes intestinaux du Renard commun *(Vulpes vulpes L.)* dans le Massif central (France). Canadian Journal of Zoology*. 66(7): 1562-1569. 10.1139/z88-228.*

Deblock, S., Pétavy, A. F., & Gilot, B. (1988). Helminthes intestinaux du Renard commun *(Vulpes vulpes L.)* dans le Massif Central (France). Canadian Journal of Zoology, *66*(7), 1562–1569.

Deplazes, P., Hegglin, D., Gloor, S. & Romig, T. (2004). Wilderness in the city: the urbanization of *Echinococcus multilocularis.* Trends in Parasitology*.* 20 (2), 77-84.

Di Cesare, A., Catagna, G., Otranto, D., Meloni, S., Milillo, P., Latrofa, M. S., Paoletti, B., Bartolini, R & Traversa, D. (2012). Molecular Detection of *Capillaria aerophila,* an Agent of Canine and Feline Pulmonary Capillariosis. Journal of Clinical Microbiology, 50 (6), 1958–1963.

Diakou, A., Karamanavi, E., Eberhard, M. & Kaldrimidou, E. (2012). First report of *Spirocerca lupi* infection in red fox Vulpes vulpes in Greece. Wildlife Biology. 18(3), 333-336.

Douch, P.G.C. & Morum, P.E. (1993).The effect of age on the response of Romney sheep to gastrointestinal nematodes during grazing. International Journal for Parasitology, 23(5), 651-655.

Duscher, G. G., Leschnik, M., Fuehrer, H. P., Joachim, A. (2015). Wildlife reservoirs for vector-borne canine, feline and zoonotic infections in Austria. Int J Parasitol Parasites Wildl. 4(1), 88– 96.

EFSA (2016). Network on Microbiological Risk Assessment Minutes of the 14th meeting. Retrieved in March 25, 2018, from EFSA: https://www.efsa.europa.eu/sites/default/files/event/160412a-m.pdf

Eira, C., Vingada, J., Torres, J., & Miquel, J. (2006*).* The Helminth Community of the Red Fox, *Vulpes Vulpes,* In Dunas de Mira (Portugal) and its effect on host condition. Wildlife Biology in Practice, *2(1), 26-36*.

Del Bove, E. & Isotti, R. (2001). The European Badger *(Meles meles)* diet in a Mediterranean area. Hystrix It. J. Mumm., 12 (1) 19 – 25.

European commission (2017). Environment: Natura 2000. Retrieved in March 28, 2018, from ec.europa.eu: http://ec.europa.eu/environment/nature/natura2000/index_en.htm

Ferrantelli, V., Riili, S., Vicari, D., Percipalle, M., Chetta, M., Monteverde,V., Gaglio, G., Giardina, G., Usai, F., Poglayen, G., 2010. *Spirocerca lupi* isolated from gastric lesions in foxes *(Vulpes vulpes)* in Sicily (Italy).Pol. J. Vet. Sci. 13, 465–471.

Figueiredo, A., Oliveira, L., Madeira de Carvalho, L., Fonseca, C., & Torres, R. T. (2016). Parasite species of the endangered Iberian wolf *(Canis lupus signatus)* and a sympatric widespread carnivore. International Journal for Parasitology: Parasites and Wildlife, *5*(2), 164–167.

Fiocchi, A., Gustinelli, A., Gelmini, L., Rugna, G., Renzi, M., & Fontana, M. C. (2016). Helminth parasites of the red fox *Vulpes vulpes* (L., 1758) and the wolf *Canis lupus italicus* Altobello *,* 1921 in Emilia-Romagna , Italy. Italian Journal of Zoology, *83*(4), 503–513.

Franssen, F., Deksne, G., Esíte, Z., Havelaar, A., Swart, A. & Van der Giessen, J. (2014). Trend analysis of *Trichinella* in a red fox population from a low endemic area using a validated artificial digestion and sequential sieving technique. Veterinary Research, 45, 120-131.

Giannelli, A., Baldassarre, V., Ramos, R.A.N, Lia, R.P., Furlanello, T., Trotta, M., Dantas-Torres, F., Baneth, G., Otranto, D. (2014). *Spirocerca lupi* infection in a dog from southern Italy: an "old fashioned" disease? Parasitology Research, 113(6), 2391-2394.

Gortázar, C., Ferroglio, E., & Höfle, U. (2007). Diseases shared between wildlife and livestock : a European perspective. Eur. J. Wildl. Res, 53, 241-256.

Gortázar, C., Villafuerte, R., Lucientes, J., Fernandez-de-Luco, D. (1998). Habitat related differences in helminth parasites of red foxes in the Ebro Valley. Vet. Parasitol., 80, 75–81.

Gottstein, B., Stojkovic, M., Vuitton, D. A., Millon, L., Marcinkute, A., Deplazes, P. (2015). Threat of alveolar echinococcosis to public health – a challenge for Europe, Trends in Parasitology, 31(9), 407 – 412.

Guberti V, Bolognini M, Lanfranchi P, Battelli G (2004). *Echinococcus granulosus* in the wolf in Italy. Parassitologia, 46, 425–427.

Guerra, D., Armua-Fernandez, M.T., Silva, M., Bravo, I., Santos, N., Deplazes, P., & Carvalho, L.M.M. (2013). Taeniid species of the Iberian wolf (*Canis lupus signatus*) in Portugal with special focus on *Echinococcus* spp. International Journal for Parasitology: Parasites and Wildlife, *2*, 50–53.

Guerra, D. R. A. (2012). The sylvatic and synanthropic cycles of *Echinococcus* spp., *Taenia* spp. e *Toxocara* spp. in Portugal: coprological and molecular diagnosis in canids. Thesis for the degree of Master of Veterinary Medicine, University of Lisbon, Lisbon, Portugal.

Guerra, D., Silva, M., Bravo, I., Valverde, A., Minas, M., Santos, N., Madeira de Carvalho, L. (2012). Wild carnivores as key hosts for the maintenance of *Toxocara* spp. in Portugal. Poster presentation in *Toxocara* 2012, Budapest, Hungary.

Haukisalmi, V., Henttonen, H. & Tenora, F. (1988). Population Dynamics of Common and Rare Helminths in Cyclic Vole Populations. The Journal of Animal Ecology, 57(3), 807.

Hegglin, D. & Deplazes, P. (2013). Control of *Echinococcus multilocularis*: Strategies, feasibility and cost–benefit analyses. International Journal for Parasitology, 43 (5), 327-337.

Hermosilla, C., Kleinertz, S., Silva, L.M.R., Hirzmann, J., Huber. D., Kusak, J., Taubert, A. (2017). Protozoan and helminth parasite fauna of free-living Croatian wild wolves *(Canis lupus)* analyzed by scat collection. Veterinary Parasitology, 233, 14–19.

Hinaidy, H. K. (1971). Die Parasitenfauna des Rotfuchses, *Vulpes vulpes (L*.), in Österreich. Zentralblatt für Veterinärmedizin Reihe B, 18: 21-32.

Hirvelä-Koski, V., Haukisalmi, V., Kilpelä, S.S., Nylund, M., Koski, P. (2003). *Echinococcus granulosus* in Finland. Vet. Parasitol. 111,175–192.

Hoberg, E. P. (2001). Out of Africa - The origins of the Tapeworm. Agricultural Research Magazine, pp.16-18

Hotez, P. J., Brooker, S., Bethony, J.M., Bottazzi, M. E., Loukas, A., Xiao, S. (2004). Hookworm Infection. N Engl J Med., 351, 799-807.

Hurníková, Z., Chovancová, B., Bartková, D., Dubinský, P. (2007). The role of wild carnivores in the maintenance of trichinellosis in the Tatras National Park, Slovakia. Helminthologia, 44(1), 18 – 20.

Ilić, T., Becskei, Z., Petrović, T., et al. (2016). Endoparasitic fauna of red foxes *(Vulpes vulpes)* and golden jackals *(Canis aureus)* in Serbia. Acta Parasitologica, 61(2), pp. 389-396.

Ilie, M. S., Imre, K., Irme, M., Sorescu, I. D., Hotea, I., Andrei, S., Florin, H., Badea, C., Morariu, S., Dărăbus, G. (2015). The prevalence of gastrointestinal parasites in red foxes *(Vulpes vulpes*) from Western Romania – Preliminary study. Scientific Works. Series C. Veterinary Medicine. Vol. LXI (1). Pp 172-176.

Játiva, P. D. C., Morgan, E. R., Barrows, M., Wronski, T. (2018). Gastrointestinal parasites in captive and free-ranging birds and potential cross-transmission in zoo environment. Journal of Zoo and Wildlife Medicine, 49(1),116-128.

Jones, A., and Walters, T.M.H. (1992). The cestodes of foxhounds and foxes in Powys, mid-Wales. Annals of Tropical Medicine and Parasitology, 86,143–150.

Kornas, S., Wierzbowska, I. A., Gorski, P., Okarma, H. (2013). Occurrence of internal parasites in stone martens (*Martes foina*) from Cracow and suburbs. Annals of Parasitology, *59*(4), 203–205.

Kruse, H., Kirkemo, A., & Handeland, K. (2004). Wildlife as Source of Zoonotic Infections. Emerging Infectious Diseases, *10*(12), 2067-2072.

Kuenzli, E., Neumayr, A., Chaney, M., & Blum, J. (2016). Acta Tropica Toxocariasis-associated cardiac diseases — A systematic review of the literature, 154, 107–120.

Lalošević, D., Lalošević, V., Klem, I., Stanojev-Jovanović, D., & Pozio, E. (2008). Pulmonary Capillariasis Miming Bronchial Carcinoma. The American Journal of Tropical Medicine and Hygiene, *78*(1), 14-16.

Lee, L. M., Wallace, R. S., Clyde, V. L., Gendron-fitzpatrick, A., Sibley, S. D., Stuchin, M., Lauck M., O'Connor, D.H., Nakao M., Lavikainen A., Hoberg E.P. & Goldberg, T. L. (2016). Definitive Hosts of *Versteria* Tapeworms (Cestoda: Taeniidae) Causing Fatal Infection in North America*, 22*(4), 707–710.

Lempp, C., Jungwirth, N., Grilo, M.L, Reckendorf, A., Ulrich, A., Neer, A. V. ,Siebert U., Bodewes R., Pfancuche V. M., Bauer C., Hosterhaus A. D. M. E., Baumgärtner W., Siebert U. (2017). Pathological findings in the red fox *(Vulpes vulpes*), stone marten *(Martes foina)* and raccoon dog *(Nyctereutes procyonoides),* with special emphasis on infectious and zoonotic agents in Northern Germany. Plos One, 12(4), 1-20.

Letková, V., Lazar, P., Soroka, J., Goldová, M. & Curlík, J. (2008). Epizootiology of game cervid cysticercosis. Nat. Croat., 17(4), 311–318.

Loos-Frank, B. & Zeyhle, E. (1982). The Intestinal Helminths of the Red Fox and Some Other Carnivores in Southwest Germany. Zeitschrift für Parasitenkunde, 67 (1), 99 -113.

Lymbery, A. J. (2017). Chapter Three - Phylogenetic Pattern, Evolutionary Processes and Species Delimitation in the Genus *Echinococcus,* Advances in Parasitology, 95,111-145.

Magalhães, A., Sousa, C. B., Afonso-Roque, M. M., Fonseca, I. M. P., Meireles, J., Fazendeiro, M. & Madeira de Carvalho, L. M. (2004). The role of wild boar and carnivores in the epidemiology of trichinellosis in Portugal. Galemys, *16*, 207–210.

Magi, M., Macchioni, F., Dell'omodarme, M., Prati, M. C., Calderini, P., Gabrielli, S., Iori, A., Cancrini, G. (2009). Endoparasites of red fox *(Vulpes vulpes)* in central Italy. Journal of Wildlife Diseases*, 45*(3), 881–885.

Magnaval, J. F., Glickman, L. T., Dorchies, P., Morassin, B. (2001). Highlights of human toxocariasis. The Korean Journal Parasitology., 39(1), 1–11.

Martínez- Carrasco, C., Ruiz de Ibanez, M. R., Sagarminaga, J. L., Garijo, M. M., Moreno, F., Acosta, I., Hernadéz, S. & Alonso, F. D. (2007). Parasites of the red fox *(Vulpes vulpes* Linnaeus, 1758) in Murcia, southeast Spain. Revue Méd. Vét., 158, 7, 331-335.

Mateus, T. L. & Barrocas, C. (2012). Wild carnivores as a source of zoonotic helminths in northern Portugal. Joint 61st WDA/ 10th Biennial EWDA conference: "Convergence in wildlife health". Book of abstracts Pp:135

Mateus, T. L., Castro, A., Ribeiro, J. N. & Vieira- Pinto, M. (2014). Multiple zoonotic parasites indentified in dog feces collected in Ponte de Lima, Portugal – A potential threat to human health. Int. J. Environ. Health, 11, 9050-9067.

Maurelli, M. P., Rinaldi, L., Alfano, S., Pepe, P., Coles, G. C. & Cringoli, G. (2014). Mini-FLOTAC, a new tool for copromicroscopic diagnosis of common intestinal nematodes in dogs. Parasites & Vectors, 7, 356 -361

Millán, J. & Ferroglio, E. (2001). Helminth parasites in stone martens *(Martes foina)* from Italy. Zeitschrift für Jagdwissenschaft, 47(3*), 229–231.*

Miquel, J., Feliu, C., Torres, J. & Casanova, J. C., (1993-1994). Corología de las especies de nematodos parásitas de carnívoros silvestres en Cataluña (NE península ibérica). Misc. Zool., 17, 49-57.

Miquel, J., Torres, J., Casanova, J.C. & Feliu, C. (1996). New data on *Ancylostoma martinezi* Miquel, Torres, Casanova et Feliu, 1994 (Nematoda: Ancylostomatidae) parasitizing *Genetta genetta* (Carnivora: Viverridae) in Spain. Helminthologia, 33(1), 43-50.

Möhl, K., Große, K., Hamedy, A., Wüste, T., Kabelitz, P., Lücker, E. (2009). Biology of *Alaria* spp. and human exposition risk to *Alaria* mesocercariae–a review. Parasitol Res.,105, 1–15.

Monsonís, G. S. (2015). Parasitofauna del zorro *(Vulpes Vulpes*) en la Comunidad Valenciana. Thesis. Facultad de veterinaria, Universidad de Murcia, Murcia, Valencia, Spain.

Murphy, T. M., O'Connell, J., Berzano, M., Dold, C., Keegan, J. D., McCann, A., Murphy, D., & Holden, N. M. (2012). The prevalence and distribution of *Alaria alata*, a potential zoonotic parasite, in foxes in Ireland. Parasitology research, 111(1), 283-290.

OIE (2012). Terrestrial Manual (May 2012). Retrieved in April 5, 2018 from OIE: http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2008/pdf/2.01.16_TRICHINELLOSIS.pdf

OIE (2018). Animal Disease Information Summaries. Retrieved in March 12, 2018, from OIE: http://www.oie.int/fileadmin/Home/eng/Media_Center/docs/pdf/Disease_cards/TRICHI-EN.pdf

Oksanen, A., Siles-Lucas, M., Karamon, J., Possenti, A., Conraths, F.J., Romig, T., Wysocki, P., Mannocci, A., Mipatrini, D., La Torre, G., Boufana, B., Casulli, A. (2016). The geographical distribution and prevalence of *Echinococcus multilocularis* in animals in the European Union and adjacent countries: a systematic review and meta-analysis. Parasites & Vectors, 9(519), 1-23

Okulewicz, A., Perec-Matysiak, A., Buńkowska, K., & Hildebrand, J. (2012). *Toxocara canis, Toxocara cati* and *Toxascaris leonina* in wild and domestic carnivores. Helminthologia, *49 (1), 3-10*.

Otranto, D., Cantacessi, C., Dantas-torres, F., Brianti, E., Pfeffer, M., Genchi, C., Guberti, V., Capelli, G. & Deplazes, P. (2015). The role of wild canids and felids in spreading parasites to dogs and cats in Europe. Part II : Helminths and arthropods. Veterinary Parasitology, *213*(1–2), 24–37.

Papadopoulos, H., Himonas, C., Papazahariadou, M. and Antoniadou-Sotiriadou, K. (1997). Helminths of foxes and other wild carnivors from rural areas in Greece. J. Helminthol., 71,227–231.

Pavlovic, I., Zoran, K. & Marija, M. (1997). The role of foxes *(Vulpes vulpes L*.) in the epizootiology and epidemiology of nematoda parasitic zoonoses. Acta veterinaria, 47 (2-3), 177-182.

Perez, J. M., & Palma, R. (2001). A new species of Felicola (Phthiraptera: Trichodectidae) from the endangered Iberian lynx: Another reason to ensure its survival. Biodiversity and Conservation, *10*, 929–937.

Pfeifer, F. (1996). Spatial distribution patterns of *Echinococcus multilocularis* (Leuckart 1863) (Cestoda: Cyclophyllidea: Taeniidae) among red foxes in an endemic focus in Brandenburg, Germany. Epidemiology and Infection, 120(1),101-109.

Pfeiffer, F., Kuschfeldt, S. & Stoye, M. (1997). [Helminth fauna of the red fox *(Vulpes vulpes* LINNE 1758) in south Sachsen-Anhalt--1: Cestodes]. DTW. Deutsche tierärztliche Wochenschrift, 104, 445-8.

Posluszny, M., Pilot, M., Goszczynski, J., Gralak, B. (2007). Diet of sympatric pine arten *(Martes martes)* and stone marten (*Martes foina)* identified by the genotyping DNA from faeces. Annals zoologici fennici, 44, 269-284.

Poulin, R. (1996). Helminth growth in vertebrate hosts: Does host sex matter?. International journal for parasitology, 26(11), 1311-1315.

Pozio, E. (2018). Trichinellosis in the European Union: Epidemiology, Ecology and Economic Impact. Parasitology Today, *14*(1), 35–38.

Pozio, E., Rinaldi, L., Marucci, G., Musella, V., Galati, F., Cringoli, G., La Rosa, G. (2009). Hosts and habitats of *Trichinella spiralis* and *Trichinella britovi* in Europe. International Journal for Parasitology, *39*(1), 71–79.

Pritt, S., Cohen, K. & Sedlacek, H. *(2012).* The Laboratory Rabbit, Guinea Pig, Hamster, and Other Rodents. Academic Press, pp. 415-446. Retrieved from: https://www.sciencedirect.com/science/article/pii/B9780123809209000158

Pullan, R. L., Smith, J. L., Jasrasaria, R., Brooker, S. J. (2014). Global numbers of infection and disease burden of soil transmitted helminth infections in 2010. Parasites & Vectors, 7(37), 1-19.

Rataj, A. V., Posedi, J., Žele, D., & Vengušt, G. (2013). Intestinal parasites of the red fox *(Vulpes vulpes)* in Slovenia. Acta Veterinaria Hungarica, *61*(4), 454–462.

Rausch, R. L. & Fay, F. H. (1988). Postoncospheral development and cycle of *Taenia polyacantha* Leuckart, 1856 (Cestoda: Taeniidae). First part. Annales de parasitologie humaine et comparée, 63, 263-77.

Regulation (EC) No. 2075/2005 of the Commission of the European Communities. Official Journal of the European Union. Retrieved in April 28, 2018, from: http://www.vetbiblios.pt/

Rentería-Solís, Z.M., Hamedy, A., Michler, F., Michler, B. A., Lücker, E., Stier, N., Wibbelt, G., Riehn, K. (2013). *Alaria alata* mesocercariae in raccoons *(Procyon lotor)* in Germany. Parasitology research, 112 (10), 3595-3600.

Reperant, L. A., Hegglin, D., Fischer, C., Kohler, L., Weber, J. M, Deplazes, P. (2007). Influence of urbanization on the epidemiology of intestinal helminths of the red fox *(Vulpes vulpes)* in Geneva, Switzerland. Parasitol Res., 101 (3), 605-611.

Richards, D. T., Harris, S., Lewis, J. W. (1995). Epidemiological studies on intestinal helminth parasites of rural and urban red foxes *(*Vulpes vulpes) in the United Kingdom. Veterinary Parasitology, 59 (1), 39-51.

Rinald, L. , Mihalca, A.D. , Cirillo, R., Maurelli, M. P.,Montesano, M., Capasso M. & Cringoli G. (2012*).* FLOTAC can detect parasitic and pseudoparasitic elements in reptiles. Experimental Parasitology, 130, 282–284.

Roddie, G., Holland, C., Stafford, P & Wolfe, A. (2008). Contamination of fox hair with eggs of *Toxocara canis.* Journal of Helminthology, 82, 293–296.

Rosalino, L.M., Torres, J. & Santos-Reis, M. (2006). *A survey of helminth infection in Eurasian badgers (Meles meles) in relation to their foraging behaviour in a Mediterranean environment in southwest Portugal*. *European Journal of Wildlife Research,* 52(3), 202–206

Saeed, I., Maddox-Hyttel, C., Monrad, J., Kapel, C. M. O. (2006). Helminths of red foxes *(Vulpes vulpes)* in Denmark, Veterinary Parasitology, 136 (1-3), 168-179.

Samuel, W. M, Pybus, M. J, Kocan, A. A. (2001). Parasitic diseases of wild mammals (2nd Ed). Iowa State University Press/ Ames

Sanmartín, M. L., Alvarez, F., Botella, H. G., Iglesias, R. Estévez, J. & López-Román, R. (1992). A scanning electron microscope study *of Toxocara genettae* Warren 1972 (Ascarididae) with data on morphometric variation. Folia Parasitologica, *35, 355-367.*

Schneider-Crease, I., Griffin, R.H., Gomery, M.A., Dorny P., Noh, J.C, Handali, S., Chastain.H. M, Wilkins, P.P., Nunn, C.L., Snyder-Mackler, N., Beehner, J.C. & Bergman, T.J. (2017*)* Identifying wildlife reservoirs of neglected taeniid tapeworms: Non-invasive diagnosis of endemic *Taenia serialis i*nfection in a wild primate population. PLOS Neglected Tropical Diseases, 11(7), e0005709.

Schoo, G., Pohlmeyer, K., Stoye, M. (1994). Zur Helminthenfauna des Steinmarders (Martes foina Erxleben 1777). Zeitschrift für Jagdwissenschaft, *40(2), 84-90.*

Segovia, J.M., Torres, J., & Miquel, J. (2004). Helminth parasites of the red fox *(Vulpes vulpes L., 1758)* in the Iberian Peninsula: An ecological study. Acta Parasitologica*, 49, 67–79.*

Silva, M., Ferreira, I.B., Guerra, D., Deplazes, P., Rio-Maior, H., Nakamura, M., Álvares, F., Santos, N., Madeira de Carvalho, L.M. (2012). Rastreio de parasitas gastrointestinais, pulmonares e musculares em canídeos domésticos e silvestres. Book of abstracts - III Congresso Ibérico do Lobo, Lugo, Espanha, p.59.

Silva, M.S.S. (2010). Rastreio de parasitas gastrintestinais, pulmonares, cutâneos e musculares em canídeos domésticos e silvestres no norte de Portugal. Thesis for the degree of Master of Veterinary Medicine, University of Lisbon, Lisbon, Portugal.

Smith, G. C., Gangadharan, B., Taylor, Z., Laurenson, M. K., Bradshaw, H., Hide, G., Craig, P. S. (2003). Prevalence of zoonotic important parasites in the red fox *(Vulpes vulpes)* in Great Britain. Veterinary Parasitology, *118*(1), 133–142.

Sobrino, R., González, L., Vicente, J., Fernández de Luco, D., Gárate, T., Gortázar, C. (2006). *Echinococcus granulosus* (Cestoda, Taeniidae) in the Iberian wolf. Parasitology research, 99, 753-756.

Sprenger, L. K., Yoshitani, U. Y., Buzatti, A. & Molento, M. B. (2018). Occurrence of gastrointestinal parasites in wild animals in State of Paraná, Brazil. Annals of the Brazilian Academy of Sciences, 90(1), 231-238.

Sréter, T., Széll, Z., Egyed, Z., & Varga, I. (2003). *Echinococcus multilocularis:* An Emerging Pathogen in Hungary and Central Eastern Europe?. Emerging Infectious Diseases, *9*(3), 384-386.

Sréter, T., Széll, Z., Marucci, G., Pozio, E. & Varga,I (2003).Extraintestinal nematode infections of red foxes *(Vulpes vulpes*) in Hungary. Veterinary Parasitology,115 (4),329-334.

Suchentrunk, F. & Sattmann, H. (1994). Prevalence of intestinal helminths in Austrian Red Foxes *(Vulpes vulpes L.).* Ann. Naturhist. Mus. Wien, 26B, 29-38.

Takeuchi-Storm, N., Al-Sabi, M., Thamsborg, S. M., & Enemark, H. L. (2015). *Alaria alata* Mesocercariae among Feral Cats and Badgers, Denmark. Emerging Infectious Diseases, *21*(10), 1872-1874.

Taylor, L. H., Latham, S. M., & Woolhouse, M. E. J. (2001). Risk factors for human disease emergence. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences, *356*(1411), 983-989. Retrieved from http://rstb.royalsocietypublishing.org/content/356/1411/983.abstract

Taylor, M. A., Coop, R. L. & Wall, R. L. (2007). Veterinary Parasitology (3rd Ed.). Blackwell Publishing Ltd., 2080pp.

Torres, J., Feliu, C., & Miquel, J. (1997). *Vigisospirura potekhina hugoti* subsp. n. (Nematoda: Spirocercidae) from Meles meles *(Carnivora: Mustelidae)* in Spain. Journal of the Helminthological Society of Washington, *64*, 106–112.

Torres, J., Miquel, J. & Motjé, M. (2001). Helminth parasites of the Eurasian badger *(Meles meles L*.) in Spain: a biogeographic approach. Parasitology Research*,* 87 (4), 259-263.

Varcasia, A., Tamponi, C., Tosciri, G., Pipia, A. P., Dore, F., Schuster, R. K., Kandil, O. M., Manunta, M. L. & Scala, A. (2015). Is the red fox *(Vulpes vulpes)* a competent definitive host for *Taenia multiceps?* Parasites & Vectors*,* 8,491.

Visser, M., Messner, C., Rehbein, S. (2011). Massive infestation with fur mites *(Lynxacarus mustelae)* of a stone marten *(Martes foina)* from Tyrol. Wiener Klinische Wochenschrift, *123 (1)*, 36–42.

Wandeler, P., Funk, S.M., Largiader, C., Gloor, S. & Breitenmoser, U. (2003). The city-fox phenomenon: Genetic consequences of a recent colonization of urban habitat. Molecular ecology*, 1*2, 647-656.

Warren, G. (1972). Two new species of *Toxocara* from viverrid hosts. Parasitology, 65, 179-187.

Webman, R. B. & Gilman, R. H (2013). Hunter's Tropical Medicine and Emerging Infectious Disease (9th Ed.). Elsevier, pp. 921-922. Retrieved from: https://www.sciencedirect.com/science/article/pii/B9781416043904001326

Webster, W. A. (1974). Records of cestodes in varying lemmings and an arctic fox from Bathurst Island, Northwest Territories. Canadian Journal of Zoology, 52(11): 1425-1426.

Willingham, A. L., Ockens, N. W., Kapel, C. M., & Monrad, J. (1996). A helminthological survey of wild red foxes *(Vulpes vulpes)* from the metropolitan area of Copenhagen. Journal of Helminthology, *70*(3), 259–263.

Wolfe, A., Hogan, S., Maguire, D., Fitzpatrick, C., Vaughan, L., Wall, D., Hayden, T.J., Mulcahy, G. (2001). Red foxes *(Vulpes vulpes)* in Ireland as hosts for parasites of potential zoonotic and veterinary significance. The Veterinary Record, *149*(25), 759–763.

World Health Organization (2017). Media centre. Echinococcosis. Retrieved April 2, 2018, from WHO: http://www.who.int/mediacentre/factsheets/fs377/en/

World Health Organization (2017). Media centre. Taeniasis/cysticercosis. Retrieved April 11, 2018, from WHO:http://www.who.int/mediacentre/factsheets/fs376/en/

Zamini, G., Khadem-erfan, M. B., & Faridi, A. (2017). *Capillaria hepatica* in *Rattus* spp . Captured in Sanandaj and Orally uninfected Balb / C Mice With Embryonated Eggs. Int. J. Basic Sci. Med*, 2*(3), 133–138.

Anexos

Anexo I - Casuística

	Raposas	Fuínhas	Texugos	Ginetas	Outros carnívoros	Javalis	Répteis	Rapinas noturnas	Rapinas diurnas	Outras aves	TOTAL
Casos clínicos presenciados											
Trauma								5	7	5	
Debilidade / desnutrição							2	1	2	2	
Atropelamento		1						5	2	3	
Colisão com estrutura								3	4	3	
Cativeiro ilegal									6	2	
Tiro / disparo								1	2	2	
Electrocussão									2		
Doença	1										
Cativeiro acidental									1	1	
Predação		1								1	
Causa desconhecida								1			
Queda do ninho								1			
Necrópsias	53	17	19	11				2	1	3	106
TOTAL	54	19	19	11			2	19	27	22	173

Anexo II - ***Relatório de Atividades***

O presente relatório de atividades é referente ao estágio curricular desenvolvido no Centro de Ecologia, Recuperação e Vigilância de Animais Selvagens (CERVAS). Este encontra-se situado no Parque Natural da Serra da Estrela, em Gouveia, e tem como principais objetivos tomar conhecimento e resolver problemas relacionados com a conservação da fauna selvagem e do seu habitat. O centro existe desde 2006, mas desde 2009 que é gerido pela associação ALDEIA e que conta com o apoio financeiro de entidades privadas como a ANA – Aeroportos de Portugal, entre outros.

A equipa, para além de desenvolver todo o processo de recolha e recuperação dos animais, tendo como objetivo final a sua devolução à natureza, também promove ações de educação ambiental, fomentando o interesse do público em geral por iniciativas ecológicas de proteção dos habitats e preservação dos ecossistemas.

Este estágio teve como objetivos principais o contacto com a realidade deste tipo de centros e a introdução à medicina da conservação. As atividades desenvolvidas foram ao encontro daquilo que é o quotidiano num hospital de fauna selvagem, nomeadamente: acompanhamento dos animais em recuperação, apoio à manutenção do centro, realização de necrópsias, saídas de campo e colaboração em projetos de educação ambiental. A casuística é apresentada no documento em excel em anexo. Para a construção da tese de mestrado integrado foram recolhidas amostras de fezes de carnívoros silvestres durante as necrópsias, e posteriormente analisadas por métodos de coprologia parasitária qualitativos e quantitativos, como será descrito na tese. Também foram recolhidos diafragmas e posteriormente analisados para a pesquisa de *Trichinella* na Universidade de Trás-os-montes e Alto Douro.

Revisão dos parasitas gastrointestinais em carnívoros selvagens na Europa

Review of the gastrointestinal parasites in wild carnivores in Europe

Ana I. Martins[1], Ricardo Brandão[2], Teresa L. Mateus*[1,3,4]

[1]Departamento de Medicina Veterinária, Escola Universitária Vasco da Gama, Coimbra, Portugal

[2]CERVAS - Centro de Ecologia, Recuperação e Vigilância de Animais Selvagens, Gouveia; Portugal

[3]Escola Superior Agrária, Instituto Politécnico de Viana do Castelo, Ponte de Lima, Portugal

[4]EpiUnit – Instituto de Saúde Pública da Universidade do Porto, Porto, Portugal

*tlmateus@gmail.com; +351966535955

Resumo

A proximidade dos animais silvestres às áreas urbanas permite um maior contacto entre estes, os animais domésticos e o Homem, aumentando a probabilidade de transmissão de doenças zoonóticas. Grande parte das doenças, nomeadamente as parasitárias, que afetam humanos têm origem em animais selvagens. Com este artigo pretendemos fazer uma revisão dos estudos existentes na Europa sobre parasitas gastrointestinais de carnívoros selvagens, nomeadamente em lobo ibérico (*Canis lupus signatus*), lobo europeu (*Canis lupus lupus*), raposa vermelha (*Vulpes vulpes),* lince ibérico (*Linx pardinus*), marta (*Martes martes*), fuinha (*Martes foina*), texugo euroasiático (*Meles meles*) e gineta (*Genetta genetta*). Para o efeito, realizamos uma pesquisa bibliográfica online, nomeadamente nos sites *PubMed* e *ResearchGate*, colocando como palavras chave de pesquisa "*parasites/parasitas*" e o nome científico dos diferentes carnívoros estudados. A maioria dos parasitas identificados correspondem a agentes potencialmente zoonóticos (ancilostomatídeos, Taeniidae, *Toxocara*, entre outros), alertando para a importância dos estudos epidemiológicos nesta área, que podem gerar evidências da necessidade de aplicação de medidas profiláticas para minimizar a disseminação destes agentes, em prol da manutenção da saúde ambiental, animal e humana.

Summary

The so-called urbanization of the wild animals allows great contact between these animals, the domestic ones and humans, increasing the probability of transmission of zoonotic diseases. Most of the diseases, namely the parasitic ones, that affect humans, come from wild animals. This study intends to review studies in Europe on gastrointestinal parasites of wild carnivores such as iberian wolf (*Canis lupus signatus*), european wolf (*Canis lupus lupus*), red fox (*Vulpes vulpes*), iberian lynx (*Linx pardinus*), marten (*Martes martes*), stone marten (*Martes foina*), eurasian badger (*Meles meles*) and common genet (*Genetta genetta*). For this purpose, we carried out an online bibliographical research, namely in the sites *PubMed* and *ResearchGate*, placing "*parasitas/parasites*" as keywords for the search and the scientific name of the different carnivores studied. Most of the

identified parasites correspond to potentially zoonotic agents (Ancylostomatidae, Taeniidae, *Toxocara*, among others). These results generate awareness on the importance of the epidemiological studies in this area, and evidence the need of applying prophylactic measures to minimize the dissemination of these parasites, protecting the environmental, animal and human health.

Introdução

As atividades antropogénicas têm-se tornado cada vez mais prejudiciais para a manutenção dos ecossistemas, e fatores como a destruição de habitats, a expansão da população humana e as alterações climáticas têm levado a uma proximidade dos animais silvestres às áreas urbanas, permitindo um maior contacto entre estes, os animais domésticos e o Homem (Carmena e Cardona, 2014; Figueiredo *et al.*, 2016). Esta evidência aliada ao facto de cerca de 61% dos agentes considerados patogénicos para o Homem serem zoonóticos, levaram à emergência do conceito *One Health*, cujo objetivo geral é atingir níveis ótimos de saúde para os animais, para o Homem e para o ambiente (Rabozzi *et al.*, 2012; OIE, 2013; Carmena e Cardona, 2014).

Sabendo que 71,8% das doenças emergentes em humanos têm origem em animais selvagens (Jones *et al.*, 2008) e tendo em conta o forte impacto que os parasitas podem ter nestes animais, os estudos de prevalência e diversidade de parasitas gastrointestinais na fauna selvagem tornam-se fundamentais, nomeadamente nos animais que se encontram em estado de conservação ameaçado, já que estará em causa não só a saúde animal e humana, mas também a biodiversidade – condição absolutamente necessária para a manutenção saudável dos ecossistemas (Carmena e Cardona, 2014; Figueiredo *et al.*, 2016).

Neste artigo pretendemos fazer uma revisão dos parasitas gastrointestinais mais prevalentes em carnívoros selvagens da Europa, com base em estudos realizados a partir de amostras coprológicas e de cadáveres de espécies como o lobo europeu

(*Canis lupus lupus*), o lobo ibérico (*Canis lupus signatus*), a raposa vermelha (*Vulpes vulpes*), o lince ibérico (*Linx pardinus*), a marta (*Martes martes*) e a fuinha (*Martes foina*), o texugo euroasiático (*Meles meles*) e a gineta (*Genetta genetta*). Para o efeito, realizamos uma pesquisa bibliográfica online, nomeadamente nos sites *PubMed* e *ResearchGate*, colocando como palavras chave de pesquisa "*parasites/parasitas*" e o nome científico dos diferentes carnívoros estudados.

Parasitas gastrointestinais no lobo europeu (*Canis lupus lupus*) e no lobo ibérico (*Canis lupus signatus*)

A população de lobo ibérico (*Canis lupus signatus*) diminuiu ao longo do século XX (em particular em Portugal), bem como a do lobo europeu (*Canis lupus lupus*) que se encontra, atualmente, em apenas algumas zonas limitadas da Europa (Figueiredo *et al.*, 2016; Hermosilla *et al.*, 2017).

Como grandes carnívoros que são, os lobos são hospedeiros de uma elevada diversidade de parasitas gastrointestinais, tendo sido relatada por Craig & Craig (2005) a presença de 72 espécies de helmintes em lobos, 93% dos quais foram identificados em necrópsias. A Tabela 1 apresenta uma compilação de estudos de prevalência de helmintes identificados em amostras fecais de lobos na Europa.

Tabela 1 - Prevalência (%) de helmintes identificados em amostras fecais de lobos (*Canis lupus*) na Europa

País	Trematodes	%	Cestodes	%	Nematodes	%	Referência Bibliográfica
Alemanha	n.i.	-	*Taenia krabbei*	77,0	n.i.	-	Lesniak *et al.* (2017)
			Taenia hydatigena	15,0			
			Mesocestoides litteratus	9,0			
Croácia	*Alaria alata*	0,3	*Taenia* spp.	1,5	Ancilostomatídeos	13,1	Hermosilla *et al.* (2017)
					Toxocara canis	2,8	
					Toxascaris leonina	0,3	
Espanha	n.i.	-	n.i.	-	Ancilostomatídeos	15,5	Pereira *et al.* (2017)
Espanha	n.i.	-	*Taenia hydatigena*	44,7	Ancilostomatídeos	71,4	Segovia *et al.* (2001)
			Taenia multiceps	29,8	*Toxocara canis*	6,4	
			Dipylidium caninum	6,4	*Toxascaris leonina*	4,8	
			Mesocestoides litteratus	4,2			
			Taenia serialis	2,1			
Polónia	n.i.	-	n.i.	-	*Trichuris vulpis*	13,9	Boreka *et al.* (2013)
					Toxocara canis	6,0	
Portugal	n.i	-	n.i.	-	*Toxocara canis*	9,09	Figueiredo *et al.* (2016)
					Toxascaris leonina	9,09	
Portugal	n.i.	-	Taeniidae	4,3	Ancilostomatídeos	45,7	Silva, 2010
			Moniezia spp.	0,6	*Strongyloides* spp.	21,3	
					Eucoleus aerophila	4,3	
					Toxocara canis	7,3	
					Toxascaris leonina	7,3	
					Trichuris spp.	3,7	
					Nematodirus spp.	0,6	

n.i. – não identificado

Tabela 1 – Prevalência (%) de helmintes identificados em amostras fecais de lobos (*Canis lupus*) na Europa (cont.)

País	Trematodes	%	Cestodes	%	Nematodes	%	Referência Bibliográfica
Portugal	n.i.	-	*Taenia hydatigena*	11,8	*Toxocara* spp.	11,8	Guerra (2012)
			Taenia serialis	5,9	Ancilostomatídeos	17,6	
			Taenia pisiformis	2,9	*Toxascaris leonina*	7,4	
			Taenia polyacantha	1,5	*Trichuris vulpis*	5,9	
			Echinococcus granulosus	1,5	*Strongyloides* sp.	1,5	
Sérvia	n.i.	-	*Taenia hydatigena*	9,8	n.i.	-	Ćirović *et al*. (2015)
			Taenia multiceps	3,9			
			Taenia polyacantha	2,9			
			Taenia taeniaeformis	2,0			
			Taenia pisiformis	1,0			
			Taenia serialis	1,0			
			Mesocestoides litteratus	1,0			

n.i. – não identificado

Os ancilostomatídeos e os ascarídeos são os parasitas mais frequentes em carnívoros selvagens de todo o mundo (Borecka *et al.*, 2013). Os ancilostomatídeos são nematodes que podem ser hematófagos ou histiófagos que parasitam o trato gastrointestinal de mamíferos como os carnívoros domésticos, carnívoros selvagens e o Homem. Em ancilostomoses severas verifica-se a ocorrência de anemia, perda de peso, fadiga, mau estado do pêlo, perda de apetite, diarreia mucosa ou hemorrágica e pode existir picacismo. Entre os ancilostomatídeos, os que ocorrem com maior frequência são *Uncinaria stenocephala* e *Ancylostoma caninum* que, associados a fatores de stress, podem ser fatais para animais jovens (Seguel e Gottdenker, 2017). *Ancylostoma caninum* é um parasita de grande interesse devido ao potencial zoonótico que apresenta. Este pode ser transmitido por via oral, galactogénea, percutânea e a ingestão de hospedeiros paraténicos também é importante na perpetuação do seu ciclo.

Os ascarídeos são de grande importância epizoótica para mamíferos predadores das famílias Canidae e Felidae (Okulewicz *et al.*, 2012). Apesar de se ter

verificado que a prevalência de *Toxocara canis* decresceu nos últimos 30 anos em cães domésticos, os níveis de infeção em cães errantes continuam elevados, a rondar os 25%. A contaminação ambiental de zonas urbanas e rurais funciona como foco de infeção para o Homem e para os animais domésticos e selvagens, crendo-se que os animais selvagens funcionem como principais reservatórios (Guerra *et al.*, 2012). A infeção pode ocorrer por ingestão dos ovos embrionados presentes no ambiente, por ingestão de tecidos de roedores contendo estádios larvares dos parasitas e, no caso de *T. canis*, por via transplacentária e galactogénica. A baixa prevalência de ascarídeos pode ser explicada devido ao reduzido número de cachorros (lobos com menos de 6 meses) nos estudos em causa, uma vez que são estes os maiores reservatórios, principalmente, da espécie *T. canis* (Hermosilla *et al.*, 2017).

A prevalência de cestodes foi baixa em países como a Polónia, a Bielorrúsia, a Sérvia e a Croácia, mas elevada na Alemanha e em Espanha.

Embora com prevalências muito baixas, esporadicamente podem aparecer ovos de trematodes como *Diphyllobothrium* spp., *Opisthorchis felineus* e *Alaria alata* nas fezes de lobos e de mustelídeos (Segovia *et al.*, 2001; Hermosilla *et al.*, 2017). A presença destas espécies de parasitas demonstra a capacidade de adaptação destes carnívoros a diferentes ecossistemas e diferentes fontes alimentares. A transmissão de *Alaria alata* aos hospedeiros definitivos (cão, gato, lobo, raposa, marta e outros carnívoros selvagens) ocorre através da ingestão do hospedeiro intermediário (molusco gastrópode) e é também facilitada pela vasta diversidade de hospedeiros paraténicos que apresenta (anfíbios, répteis, aves e mamíferos) (Cordero del Campillo *et al.*, 1999). O género *Diphyllobothrium* apresenta um primeiro hospedeiro intermediário - crustáceos (Diemert, 2017) e pequenos invertebrados aquáticos (Kelly e Mutengo, 2017) que funciona como fonte de infeção para o segundo hospedeiro intermediário (peixes anádromos e de água salgada) (Diemert, 2017). Já *Opisthorchis felineus* tem como hospedeiros intermediários gastrópodes e peixes de água doce da família Cyprinidae (Jones, 2015).

Comparando com os helmintes, há um número mais reduzido de estudos sobre a prevalência de protozoários em carnívoros selvagens. Contudo, num estudo recente realizado na Alemanha, foram identificadas 12 espécies do género *Sarcocystis* em carcaças de lobos com uma prevalência total de 95% (Lesniak *et al.*, 2017). Este parasita apresenta um ciclo de vida indireto, reproduz-se sexualmente no intestino do carnívoro, que funciona como hospedeiro definitivo, e a forma infetante é libertada para o ambiente com as fezes. Esta é, depois, ingerida pelo hospedeiro intermediário onde invade o tecido muscular cardíaco e esquelético. A transmissão ocorre quando o carnívoro ingere a presa, normalmente herbívoros, como os cervídeos e o javali, contendo os quistos musculares com os bradizoítos (Lesniak *et al.*, 2017). Na Croácia, a prevalência foi mais baixa (19,1%) (Hermosilla *et al.*, 2017), e em Portugal, ainda que com prevalências ainda mais baixas, duas espécies são referidas por Silva (2010), *Sarcocystis canis* (7,9%) e *Sarcocystis felis* (0,6%). As espécies dos géneros *Cryptosporidium* e *Giardia* parecem ser menos frequentes, o que, e em relação a *Cryptosporidium*, se pode dever ao facto de ser mais difícil recolher amostras de fezes de crias de lobo (Hermosilla *et al.*, 2017). Em Espanha, Pereira *et al.* (2017) reportam, contudo, uma prevalência de 20% em amostras de lobo ibérico para os géneros *Giardia* e *Cryptosporidium*, o que revela a suscetibilidade destes animais a estes agentes, assim como o seu potencial papel enquanto disseminadores dos mesmos. Mateo *et al.* (2017), também em Espanha, identificaram *Giardia duodenalis* numa amostra de lobo ibérico. Em Portugal, Silva (2010) identificou os géneros *Cryptosporidium* (13,5%), *Eimeria* (4,9%) e *Cystoisospora* (3,7%).

Parasitas gastrointestinais em raposas vermelhas (*Vulpes vulpes*)

A raposa vermelha (*Vulpes vulpes*) é um carnívoro selvagem cuja população se encontra em expansão e está bem distribuída a nível global. Este carnívoro é a espécie selvagem mais estudada, devido à sua proximidade às áreas urbanas e à facilidade de recolher amostras fecais que lhe pertençam (Borecka *et al.*, 2013). No entanto, em Portugal, em particular na região a Sul do rio Douro, os estudos parasitológicos que a envolvem são escassos (Figueiredo *et al.*, 2016). Enquanto

espécie sinantrópica, a raposa permite a ligação entre as áreas silvestres e zonas urbanas, o que aumenta a probabilidade de transmissão de zoonoses entre animais selvagens e domésticos e o Homem (Figueiredo *et al.*, 2016). A Tabela 2 apresenta uma compilação de estudos de prevalência de helmintes identificados em amostras fecais de raposas vermelhas (*Vulpes vulpes*) na Europa.

Tabela 2 – Prevalência (%) de helmintes identificados em amostras fecais de raposas vermelhas (*Vulpes vulpes*) na Europa.

Países	**Trematodes**	**%**	**Cestodes**	**%**	**Nematodes**	**%**	**Referências bibliográficas**
Croácia	n.i.	-	*Echinococcus multilocularis*	7,2	n.i.	-	Beck *et. al.* (2018)
Eslovénia			*Mesocestoides* spp.	27,6	*Uncinaria stenocephala*	58,9	Rataj *et al.* (2013)
			Taenia crassiceps	22,2	*Toxocara canis*	38,3	
			Taenia polyacantha	6,5	*Toxascaris leonina*	2,5	
			Taenia pisiformis	2,1	*Trichuris vulpis*	0,7	
			Dypilidium caninum	1,4			
			Echinococus multilocularis	2,6			
Grã-Bretanha	n.i.	-	n.i.	-	*Toxocara canis*	61,1	Smith *et al.* (2003)
					Uncinaria stenocephala	41,3	
Grécia	n.i.	-	n.i.		*Uncinaria stenocephala*	43,9	Papadopoulos *et al.* (1997)
					Toxocara canis	28,6	
Irlanda	n.i	-	n.i.	-	*Capillaria aerophila*	26,0	Stuart et al., 2013
					Toxocara canis	20,0	
					Trichuris vulpis	4,0	
					Uncinaria stenocephala	38,0	
Itália	n.i.	-	*Dypilidium caninum*	57,3	n.i.	-	Magi *et al.* (2009)
Itália	n.i.	-	*Echinococcus multilocularis*	9,2	n.i.	-	Casulli *et al.* (2005)
Letónia	n.i.	-	*Echinococcus multilocularis*	17,1	n.i.	-	Bagrade *et al.* (2016)
Polónia	n.i.	-	n.i.	-	*Trichuris vulpis*	64,4	Borecka *et al.* (2013)
Portugal	n.i.	-	n.i.	-	*Toxocara canis*	15-40	Guerra *et al.* (2012)
Portugal	n.i.	-	n.i.	-	*Toxocara canis*	37,1	Eira *et al.* (2006)
Portugal	Trematodes	20,9	*Taenia polyacantha*	3,0	Ancilostomatídeos	43,5	Guerra (2012)
			Taenia hydatigena	3,0	*Capillaria* sp.	6,4	
			Mesocestoides spp.	1,6	*Physaloptera* sp.	4,84	
			Hymenolepis spp.	1,6	*Strongyloides* sp.	4,8	
					Trichuris vulpis	3,2	
					Toxascaris leonina	3,2	
					Spirocerca sp.	1,6	
Portugal	n.i.	-	*Anoplocephala* spp.	1.2	*Strongyloides* sp.	42,0	Silva, 2010
			Paranoplocephala spp.	1.2	Ancilostomatídeos	64,2	
					Toxocara canis	24,7	
					Eucoleus aerophilus	3,7	
					Trichuris spp.	2,5	
					Toxascaris leonina	1,2	

Suíça	n.i.	-	n.i.	-	*Toxocara canis*	14,0	Okulewicz *et al.* (2012)
					Toxascaris leonina	14,0	

n.i. – não identificado

As raposas vermelhas são vistas como principal veículo de disseminação de ascarídeos devido ao elevado número de hospedeiros paraténicos ingeridos por esta espécie (Guerra *et al.*, 2012). Dentro deste grupo, as espécies *Toxocara canis* e *Toxascaris leonina* são parasitas muito importantes na medida em que têm carácter zoonótico.

Os ancilostomatídeos caracterizam-se por ser hematófagos, provocando anemia e morte neonatal em cães e gatos domésticos. Dado o seu caracter zoonótico, revestem-se de elevada importância a nível médico e veterinário, uma vez que podem ser transmitidos não só a animais domésticos como ao Homem (Zajac e Conboy, 2012).

O aumento da população de raposas que se verificou devido ao sucesso dos programas de vacinação contra a raiva permitiu uma expansão na presença do cestode *Echinococcus multilocularis* em várias capitais europeias da Europa central e de leste, uma vez que este animal funciona como o principal hospedeiro definitivo do agente (Brossard *et al.*, 2007; Bagrade *et al.*, 2016). *Echinococcus* é um género de cestode da família Taeniidae, de carater zoonótico e com distribuição mundial. Na sua forma adulta, o parasita encontra-se no intestino de canídeos (cães domésticos, chacais, lobos, raposas, guaxinins), de felídeos (leões, pumas e jaguares) e de hienas, e os ovos saem para o exterior com as fezes. Os roedores e ungulados ingerem os ovos do ambiente e funcionam como hospedeiro intermediário, desenvolvendo o metacestode. Os humanos podem também ingerir os ovos e desenvolver hidatidose (*Echinococcus granulosus*) ou equinococose alveolar (*Echinococcus multilocularis*). Apesar da elevada prevalência que *Echinococcus multilocularis* apresenta em carnívoros selvagens, a ocorrência em gatos e cães domésticos na Europa é rara (Brossard *et al.*, 2007). Esta é uma doença grave e de elevada mortalidade em humanos, e de disseminação lenta em

grande parte da Europa continental (Learmount *et al.*, 2012), mas que pode contribuir para aumentar a ocorrência de ciclos sinantrópicos entre os cães domésticos e os roedores silváticos, aumentando a probabilidade de transmissão deste parasita ao Homem. Comparando com *Echinococcus multilocularis*, a informação acerca de *Echinococcus granulosus* é escassa. O ciclo silvático de *Echinococcus granulosus* foi documentado apenas num limitado número de países da Europa, como a Finlândia (Hirvelä-Koski *et al.*, 2003), Itália (Guberti *et al.*, 2004), Bulgária (Breyer *et al.*, 2004), Espanha (Sobrino *et al.*, 2006) e Portugal (Guerra *et al.*, 2013).

No que diz respeito a protozoários há pouca informação nesta espécie de carnívoro, no entanto, em Portugal existe um estudo onde se verificou a presença dos géneros *Cryptosporidium* (22,1%) , *Eimeria* (11,1%) e *Cystoisospora* (1,2%), assim como da espécie *Sarcocystis canis* (1,2%) (Silva, 2010). *Giardia duodenalis* e o género *Cryptosporidium* , ambos com uma prevalência de 8%, foram reportados em Espanha por Mateo *et al*. (2017) e na Eslovénia foram identificados os géneros *Sarcocystis* (2,8%) e *Cystoisospora* (0,4%) (Rataj *et al.*, 2013).

Parasitas gastrointestinais em linces ibéricos (*Linx pardinus*)

O lince ibérico é o felídeo mais ameaçado do mundo, segundo o *International Union for the Conservation of Nature* (IUCN), e os estudos parasitológicos nesta espécie são igualmente raros. Na nossa revisão identificamos dois estudos dos mesmos autores, que reportam a ocorrência de *Ancylostoma tubaeforme, Toxocara cati, Joyeuxiella pasqualei*, *Taenia polyacantha* e *Mesocestoides* sp. em linces ibéricos de Espanha (Milan e Casanova, 2007). Vicente *et al.* (2003), também em Espanha enfatizam a elevada prevalência de *Ancylostoma* spp. (57,8%) nestes animais, e referem a sua elevada prevalência e carga sobretudo em animais jovens, que poderão ser mais suscetíveis a este agente. Millan e Blanco-Costa (2012)

notam mesmo que os nematodes do género *Ancylostoma* são parasitas muito patogénicos para felídeos jovens e que *A. tubaeforme* pode causar morbilidade e mortalidade elevadas no lince ibérico.

Parasitas gastrointestinais em mustelídeos

Os estudos com mustelídeos são raros, identificamos apenas quatro, um em Portugal, um na Polónia e dois em Espanha. Os mustelídeos, alimentam-se essencialmente de roedores, apesar de também fazerem parte da sua dieta aves, répteis, insetos e frutos. A marta encontra-se distribuída por toda a Europa continental (Segovia *et al*., 2007). Borecka *et al.* (2013) na Polónia, identificaram os seguintes parasitas em martas: *Trichuris* spp. (40,0%), *Toxocara cati* (13,3%), *Ancylostoma* spp. (13,3%) e *Uncinaria* spp. (6,7%). Em Espanha, Segovia *et al.* (2007) referem *Taenia martis* (5,88%) e *Uncinaria criniformis* (0,98%), sendo que ambos os parasitas apresentam elevada especificidade. Ainda em Espanha, Mateo *et al.* (2017) reporta a ocorrência de *Giardia duodenalis* numa amostra de fuinha (*Martes foina*). Num estudo realizado recentemente em Portugal, *Crenosoma vulpis* foi identificado com elevada prevalência (31%), seguido do género *Toxocara* (15%) (Figueiredo *et al.*, 2018). Neste mesmo estudo, os ancilostomatídeos, e o género *Strongyloides* também foram identificados com uma prevalência de 8%, bem como a espécie *Toxascaris leonina* (Figueiredo *et al*., 2018).

O texugo europeu é dentro da família dos mustelídeos, a espécie mais estudada, mas ainda assim, no que diz respeito a parasitas os dados são escassos. Stuart *et al.* (2013) refere a identificação de *Uncinaria criniformis,* com uma prevalência de 40%, e *Cystoisospora* sp. com uma prevalência de 16%. Mateo *et al.* (2017) reporta a identificação de *Crysptosporidium* sp. (3%).

Parasitas gastrointestinais em ginetas (*Genetta genetta*)

Os estudos com ginetas também não são muito frequentes. Casanova *et al.* (2000) realizaram um estudo em Espanha com um número de amostras considerável (n=299) e identificaram uma grande diversidade de helmintes: *Brachylaima* sp., *Metorchis albidus*, *Taenia parva*, *Mesocestoides* sp., *Joyeuxiella pasqualei*, *Diplopylidium monoophorum*, *D. triseriale*, *Trichinella* sp., *Ancylostoma martinezi*, *Toxocara canis*, *T. genettae*, *Spirura* sp., *Cyathospirura seurati*, *Mastophorus muris*, *Physaloptera* sp., *Pterygodermatites affinis* e *P. leiperi*. Também em Espanha, Mateo *et al.* (2017), identificaram *Cryptosporidium* sp. em amostras desta espécie. Em Portugal, Mateus e Barrocas (2012) reportaram *Toxocara* (60,7%) e ancilostomatídeos (46,4%), em amostras de gineta do norte do país.

Considerações finais

A diversidade de resultados obtidos pelos diferentes estudos, quer no distinto número de espécies de parasitas identificados, quer nas suas prevalências, destaca a importância da promoção e desenvolvimento de estudos à escala local, já que as dietas destes animais variam consoante os ecossistemas em que estão inseridos, o que determina fortemente o risco de infecção parasitária. Não alheio à diversidade de resultados deverá também ser o muito variável tamanho da amostra – desde estudos com menos de 20 amostras até os que têm mais de 500 -, assim como a origem das mesmas (ambiental ou recolha em necrópsias). Finalmente, a dificuldade maior na comparação de resultados, passa também pela diversidade de métodos coprológicos usados pelos diferentes estudos (desde técnicas de flutuação com diferentes soluções, técnicas de sedimentação, técnicas de biologia molecular, entre outras).

Dado o forte impacto das parasitoses na conservação de espécies selvagens e na manutenção dos ecossistemas, e sabendo que existe um elevado risco de

transmissão destas doenças para as espécies domésticas e para o Homem devido à conhecida urbanização dos animais selvagens, torna-se necessário desenvolver estudos epidemiológicos, principalmente nas espécies sinantrópicas.

No caso dos lobos, as parasitoses podem reduzir drasticamente o número de indivíduos em populações isoladas, mesmo em áreas geográficas protegidas, pelo que, o desenvolvimento de estudos que permitam um melhor conhecimento acerca do parasitismo é essencial, pois só assim se podem aplicar ações corretivas e profiláticas que contribuirão para a sua conservação.

A consciencialização de que existe uma elevada probabilidade de disseminação de doenças zoonóticas em áreas cosmopolitas deve funcionar como catalisador para o desenvolvimento de mais estudos na área da parasitologia, uma vez que só aprofundando o conhecimento acerca da epidemiologia do parasita é que é possível proteger a saúde ambiental, animal e humana, ou seja, no âmbito de Uma Saúde.

Bibliografia

Bagrade G, Deksne G, Ozolina Z, Howlett SJ, Interisano M, Casulli A, Pozio E (2016). *Echinococcus multilocularis* in foxes and raccoon dogs: an increasing concern for Baltic countries. Parasites & Vectors, 9 (1), 615.

Beck R, Mihaljević Ž, Brezak R, Bosnić S, Janković IL, Deplazes P (2018). First detection of *Echinococcus multilocularis* in Croatia. Parasitology Research, 117 (2), 617–621.

Borecka A, Gawor J, Zieba F (2013). A survey of intestinal helminths in wild carnivores from the Tatra National Park, southern Poland. Annals of Parasitology, 59 (4), 169–72.

Breyer I, Georgieva D, Kurdova R, Gottstein B (2004). *Echinococcus granulosus* strain typing in Bulgaria: the G1 genotype is predominant in intermediate and definitive wild hosts. Parasitol. Res. 93, 127–130.

Brossard M, Andreutti C, Siegenthaler M (2007). Infection of red foxes with *Echinococcus multilocularis* in western Switzerland. Journal of Helminthology, 81 (4), 369–376.

Carmena D, Cardona GA (2014). Echinococcosis in wild carnivorous species: Epidemiology, genotypic diversity, and implications for veterinary public health. Veterinary Parasitology, 202 (3 – 4), 69–94.

Casanova JC, Fliu C, Miquel J, Torres J, Spakulová M (2000). Faunistic and ecological trends on the helminthic community of *Genetta genetta* Linnaeus, 1758 (Carnivora: Viverridae) in the Iberian Peninsula. Helminthologia, 37(4), 223-228.

Casulli A, Manfredi MT, La Rosa G, Di Cerbo AR, Dinkel A, Romig T, Pozio E (2005). *Echinococcus multilocularis* in red foxes (*Vulpes vulpes*) of the Italian Alpine region: is there a focus of autochthonous transmission? International Journal for Parasitology, 35 (10), 1079 –1083.

Ćirović D, Pavlović I, Penezić A (2015). Intestinal helminth parasites of the grey wolf (Canis lupus L.) in Serbia. Acta Veterinaria Hungarica, 63 (2), 189 –198.

Cordero del Capillo M, Vazquez FAR, Fernandez ARM, Acedo MCS, Rodriguez SH, Lopez-Cozar IN, Bañoz PD, Romero HQ, Varela MC (1999). *Parasitologia veterinaria* (1st Ed.). Mc-Graw Hill Interamericana de España, S. A. U.

Craig HL, Craig PS (2005). Helminth parasites of wolves (*Canis lupus*): A species list and an analysis of published prevalence studies in Nearctic and Palaearctic populations. Journal of Helminthology, 79, 95–103.

Diemert DJ (2017). Cestode and Trematode Infections. Infectious diseases (4th edition), 2(114), 1032-1037.

Eira C, Vingada J, Torres J, Miquel J (2006). The Helminth Community of the Red Fox, *Vulpes vulpes*, In Dunas de Mira (Portugal) and its effect on host condition. Wildlife Biology in Practice, 2.

Figueiredo A, Oliveira L, Madeira de Carvalho L, Fonseca C, Torres RT (2016). Parasite species of the endangered Iberian wolf (*Canis lupus signatus*) and a sympatric widespread carnivore. International Journal for Parasitology: Parasites and Wildlife, 5 (2), 164–167.

Figueiredo, A, Oliveira, L, de Carvalho, LM, Fonseca, C, Torres, RT (2018). Helminth parasites of stone marten (*Martes foina*) in central Portugal. Annals of parasitology, 64(1), 65-68.

Guberti V, Bolognini M, Lanfranchi P, Battelli G (2004). *Echinococcus granulosus* in the wolf in Italy. Parassitologia 46, 425–427.

Guerra, DRA (2012). The sylvatic and synanthropic cycles of *Echinococcus* spp., *Taenia* spp. and *Toxocara* spp. in Portugal: coprologic and molecular diagnosis in canids. Dissertação de mestrado em Medicina Veterinária. Universidade técnica de Lisboa, Faculdade de Medicina Veterinária, Lisboa, Portugal.

Guerra D, Silva M, Bravo I, Valverde A, Minas M, Santos N, Madeira de Carvalho L (2012). Wild carnivores as key hosts for the maintenance of *Toxocara* spp. in Portugal. Poster presentation in *Toxocara* 2012, Budapest, Hungary.

Guerra D, Armua-Fernandez MT, Silva M, Bravo I, Santos N, Deplazes P, & Carvalho LMM (2013). Taeniid species of the Iberian wolf (*Canis lupus signatus*) in Portugal with special focus on *Echinococcus* spp. *International Journal for Parasitology: Parasites and Wildlife*, *2*, 50–53.

Hermosilla C, Kleinertz S, Silva LMR, Hirzmann J, Huber D, Kusak J, Taubert A (2017). Protozoan and helminth parasite fauna of free-living Croatian wild wolves (Canis lupus) analyzed by scat collection. Veterinary Parasitology, 233, 14–19.

Hirvelä-Koski V, Haukisalmi V, Kilpelä SS, Nylund M, Koski P (2003). *Echinococcus granulosus* in Finland. Vet. Parasitol. 111,175–192.

Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL & Daszak P (2008). Global trends in emerging infectious diseases. Nature 451, 990–993.

Jones SRM (2015). Transmission dynamics of foodborne parasites in fish and shellfish. Foodborne parasites in the Food Suply Web – Occurrence and control, a volume in Woodhead Publishing Series in Food Science, Technology and Nutrition, 293-315.

Kelly P, Mutengo M (2017). Parasitic infections of the Gastrointestinal Tract. Infectious diseases (4th edition), 2(114), 989-1001.

Learmount J, Zimmer IA, Conyers C, Boughtflower VD, Morgan CP, Smith, GC (2012). A diagnostic study of *Echinococcus multilocularis* in red foxes (*Vulpes vulpes*) from Great Britain. Veterinary Parasitology, 190(3), 447–453.

Lesniak I, Heckmann I, Heitlinger E, Szentiks CA, Nowak C, Harms V, Krone O (2017). Population expansion and individual age affect endoparasite richness and diversity in a recolonising large carnivore population. Scientific Reports, 7, 41730: 1 – 14.

Magi M, Macchioni F, Dell'Omodarme M, Prati MC, Calderini P, Gabrielli S, Iori A., Cancrini G. (2009). Journal of Wildlife Diseases: Endoparasites of Red Fox (*Vulpes vulpes*) in Central Italy 45, 881-885.

Mateo M, Hernández M, Mingo D, de Lucio A , Morales L, Balseiro A, Carmena D (2017). Occurrence and molecular genotyping of *Giardia duodenalis* and *Cryptosporidium* spp. in wild mesocarnivores in Spain. Veterinary Parasitology, 235, 86–93.

Mateus TL, Barrocas C (2012). Wild carnivores as a source of zoonotic helminths in the northern of Portugal. Book of abstracts – Joint 61th WDA/10th Biennial EWDA conference convergence in wildlife health. Lyon, France, p.135.

Millán J, Blasco-Costa I (2012). Molecular evidence of shared hookworm *Ancylostoma tubaeforme* haplotypes between the critically endangered Iberian lynx and sympatric domestic cats. Veterinary Parasitology, 186 (3–4), 518–522.

Millán J, Casanova JC (2007). Helminth parasites of the endangered Iberian lynx (*Lynx pardinus*) and sympatric carnivores. Journal of Helminthology 81, 377–380.

OIE (2013). Bulletin 2013 "The One Health concept: the OIE approach". Acedido a 25 de Maio de 2018, em OIE: http://www.oie.int/fileadmin/Home/eng/Publications_%26_Documentation/docs/pdf/bulletin/Bull_2013-1-ENG.pdf

Okulewicz A, Perec-Matysiak A, Buńkowska K, Hildebrand J (2012). *Toxocara canis*, *Toxocara cati* and *Toxascaris leonina* in wild and domestic carnivores. Helminthologia, 49 (1), 3-10.

Papadopoulos H, Himonas C, Papazahariadou M, Antoniadou-Sotiriadou K (1997). Helminths of foxes and other wild carnivores from rural areas in Greece. Journal of helminthology, 71 (3), 227-231.

Pereira AL, Mateus TL, Llaneza L, Duarte SC (2017). Report of *Giardia* spp. and *Cryptosporidium* spp. in Iberian Wolf (*Canis lupus signatus*) in NW of Iberian Peninsula. Xth International Symposium on Wild Fauna. Book of abstracts. Pp: 44.

Rabozzi G, Bonizzi L, Crespi E, Somaruga C, Sokooti M, Tabibi R, Colosio C (2012). Emerging Zoonoses: the "One Health Approach." *Safety and Health at Work*, *3*(1), 77–83.

Rataj AV, Posedi J, Žele D, Vengušt G (2013). Intestinal parasites of the red fox (*Vulpes vulpes*) in Slovenia. Acta Veterinaria Hungarica, 61 (4), 454– 462.

Segovia JM, Torres J, Miquel J, Llaneza L, Feliu C (2001). Helminths in the wolf, *Canis lupus*, from north-western Spain. Journal of Helminthology, 75, 183–192.

Segovia JM, Torres J, Miquel J, Sospedra E, Guerrero R, Feliu C (2007). Analysis of helminth communities of the pine marten, *Martes martes*, in Spain: Mainland and insular data. Acta Parasitologica, 52 (2), 156 –164.

Seguel M, Gottdenker N (2017). The diversity and impact of hookworm infections in wildlife. International Journal for Parasitology: Parasites and Wildlife, 6 (3), 177–194.

Silva M, Ferreira IB, Guerra D, Deplazes P, Rio-Maior H, Nakamura M, Álvares F, Santos N, Madeira de Carvalho LM (2012). Rastreio de parasitas gastrointestinais, pulmonares e musculares em canídeos domésticos e silvestres. Book of abstracts - III Congresso Ibérico do Lobo, Lugo, Espanha, p.59.

Silva, MSS (2010). Rastreio de parasitas gastrintestinais, pulmonares, cutâneos e musculares em canídeos domésticos e silvestres no norte de Portugal. Dissertação de mestrado em Medicina Veterinária. Universidade técnica de Lisboa, Faculdade de Medicina Veterinária, Lisboa, Portugal.

Smith GC, Gangadharan B, Taylor Z, Laurenson MK, Bradshaw H, Hide G, Craig PS (2003). Prevalence of zoonotic important parasites in the red fox (*Vulpes vulpes*) in Great Britain. Veterinary Parasitology, 118 (1), 133–142.

Sobrino R, González L, Vicente J, Fernández de Luco D, Gárate T, Gortázar C (2006). *Echinococcus granulosus* (Cestoda, Taeniidae) in the Iberian wolf. Parasitology research, 99, 753-756.

Stuart P, Golden O, Zintl A, De Waal T, Mulcahy G, McCarthy E, Lawton C (2013). A coprological survey of parasites of wild carnivores in Ireland. Parasitology Research, 112 (10), 3587–3593.

Vicente J, Palomares F, Ruiz de Ibañez R, Ortiz J (2004). Epidemiology of *Ancylostoma* spp. in the endangered Iberian lynx (*Lynx pardinus*) in the Doñana National Park, south-west Spain. Journal of helminthology, 78, 179-183.

Zajac, AM, Conboy, GA (2012). *Veterinary clinical parasitology* (8th ed.). United Kingdom: Wiley-Blackwell.